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MORPHOLOGY OF THE HEAD CAPSULE AND MOUTH PARTS OF *ANTHIA SEXGUTTATA* HO. (COLEOPTERA, ADEPHAGA, CARABIDAE, HARPALINAE, ANTHIINI)

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I. Introduction

The genus *Anthia* (Pachymorpha) is represented in India by a single large species, *Anthia sexguttata* Ho. It is one of the most stricking black beetles with six large white spots. Lefroy (1909) has reported that the species belongs to the plains, but it has also been locally collected in the hilly tracts of Ajmer (1,581 ft. above M.S.L.) by one of us (N. K.). The insect is predacious in habits and feeds on other small insects including grasshoppers.

General accounts dealing with the head capsule and mouth parts of the family Carabidæ are available by Stickney (1923) and Williams (1938). They

have discussed the phylogeny of the Coleoptera and are of the opinion that Carabidæ is the most primitive group. However, hardly any contribution has been made towards the study of the morphology of the head capsule and mouth parts of Anthiini. Hence, we have studied the mouth parts and head capsule of *Anthia sexguttata* Ho.

II. MATERIAL AND METHOD

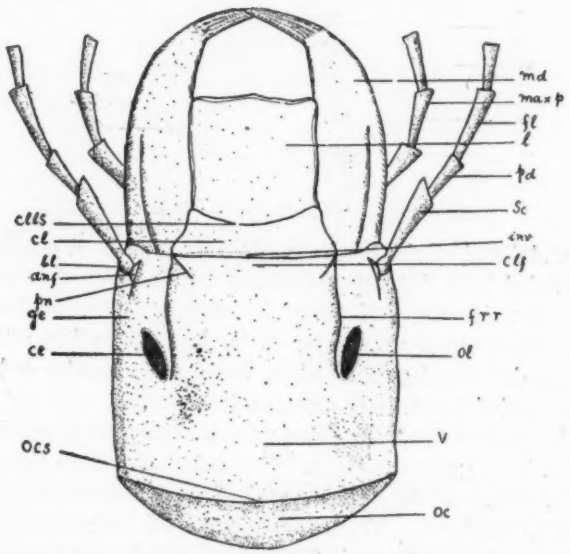
The adult beetles were locally collected in the months of August and September and were identified as *Anthia sexguttata* Ho. (Lefroy, 1909). They were killed in potassium cyanide bottle and preserved in 70% alcohol. The head capsule and mouth parts were made transparent by boiling in 15% potassium hydroxide. The sclerites were stained in 1% solution of picro-indigo-carmine and the various structures were studied under a dissecting binocular microscope.

III. HEAD CAPSULE

The much chitinised head capsule of *Anthia sexguttata* Ho. is almost rectangular in shape with its posterior margin convexly curved. It is slightly broader than long and occupies a horizontal position in front of the thorax. The mouth parts are prognathous and are directed cephalad. The occipital foramen lies opposite to the mouth at the caudal end of the head.

The epicranial stem is absent in *Anthia sexguttata* and the epicranial arms, as in *Harpalus* (Stickney, 1923), are in the form of a ridge along the edge of an invagination which is wedge-shaped and runs horizontally across the dorsal surface of the head between the bases of the gnathal appendages. This invagination represents the epistomal or frontoclypeal suture. Stickney (1923) while describing the head capsule in Coleoptera mentions that "there is no external indication in any head of a frontoclypeal suture." At the ends of the epistomal suture are present two short and oblique furrows, the pretentorinæ, which lead into the respective anterior tentorial arm. The pretentorinæ lie at the ends of the epicranial arms. Between the epicranial arms is the frons. Thus the invagination assumes a compound nature by having in it the entire frons, the epicranial arms, and forming the anterior and posterior boundaries of the vertex and the clypeus respectively. Stickney (1923) has termed that part of the invagination which lies between the pretentorinæ as the clypofrons. The dorsal surface of the head upto the occiput, and separated from it by the occipital suture, is the vertex which has a smooth dorsal surface. Because of the peculiar position of the epicranical arms and the compound nature of the invagination, the frons is reduced, a clypofrons is formed, and the vertex is much increased in extent. On the midlateral sur-

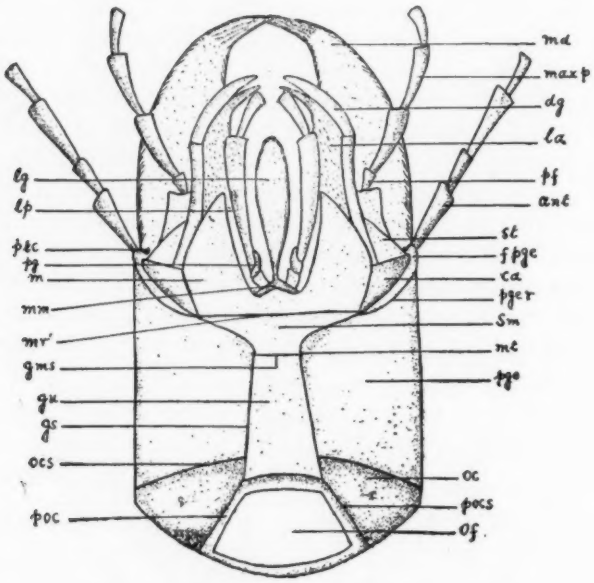
face of the vertex, on either side, is present a compound eye. Each compound eye is small, oval and surrounded by a narrow sclerite, the oculata. The anterolateral area of the vertex below the compound eye is the gena. The genæ are separated from the vertex mesially by a well developed frontal ridge. The frontal ridge extends from the invagination and arches over the mesial margin of the compound eye. There is no trace of any ocellus in *A. sexguttata* as is the case in other members of the group Coleoptera. Each gena bears an antenna at its cephalic end. The antennæ are markedly kataceratous (Crampton, 1923). Each antenna is a short four-segmented structure, and fits into a depression, the antafossa, on the dorsal surface of the gena. The antafossa is surrounded by an antennal sclerite, the antennarium, with which the antenna movably articulates by means of a membrane, the antacorium. The basal segment of the antenna, known as scape, is the longest of all. The second segment, the pedicel, is shorter than the scape. The third and fourth segments form the flagellum. The base of the scape is bulb-like and fits into the antafossa. The base of each antenna possesses two short tendons, the antatendons, for the attachment of the muscles that bring about its movement.



TEXT-FIG. 1. Dorsal view of the head capsule.

A prominent feature of the head capsule of *A. sexguttata* is the presence of a well-developed occiput separated from the postgena and the vertex by

an occipital suture on the ventral and the dorsal surfaces respectively. The occipital suture arises laterad of the gula on the ventral side of the head, and runs obliquely caudad extending to the dorsal surface where it joins its fellow on the meson.



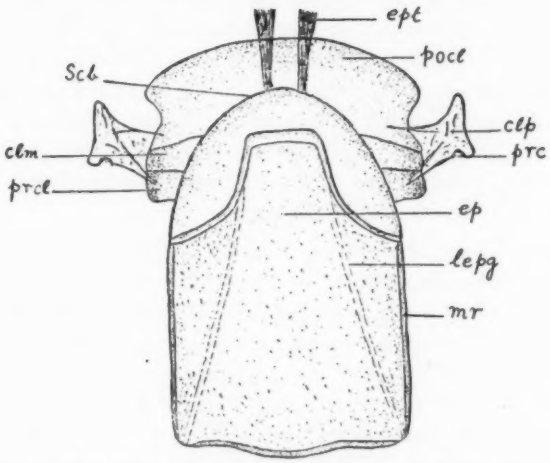
TEXT-FIG. 2. Ventral view of the head capsule.

The caudal end of the head capsule is formed by a small occipital foramen surrounded by a distinct postocciput which articulates with the cervix. The postocciput is separated from the occiput by a postoccipital suture. Anterior to the postocciput and on the midventral surface of the head capsule is a well-developed gula which is broad at its caudal end. Laterad the gula is surrounded by the occiput and the postgena, marked off from each other by the occipital suture. The gula is separated from the occiput and the postgena by a gular suture on either side, and from the submentum by a gulomental suture at the cephalic end. The postgenæ are large areas with smooth dorsal surfaces. The cephalic part of each postgena is modified into a flat area sloping towards the meson, which at its mesial end bears a cavity, the paracoila, for the articulation of the cardo of the maxilla. The paracoilæ lie beneath the cephalolateral ends of the submentum. On the cephalic end of the meson there is another cavity, the postcoila, for the articulation of the postartis of the mandible of the respective side. The flat

area of the postgena is separated from the rest of it by a distinct ridge, the postgenal ridge, which curves from the lateral margin of the postcoila to the caudolateral margin of the submentum. At each caudolateral end of the submentum is the metatentorina which leads into the posterior tentorial arm.

IV. Mouth Parts

(*a*) *Clypeolabrum.*—The clypeolabrum occupies the anterodorsal area of the preoral cavity. Its dorsal surface is slightly convex. Separated from the vertex by a distinct invagination, the epistomal or frontoclypeal suture, is the postclypeus. The cephalic end of the postclypeus is infolded and carries the preclypeus on its ventral surface. Thus the preclypeus cannot be seen in the dorsal view unless the postclypeus is much stretched in the anterior direction. Between the pre- and post-clypeus there is a small membrane, the clypeal membrane. The preclypeus is small, less chitinised, rectangular



Text-Fig. 3. Ventral view of the clypeolabrum.

and alutaceous. The postclypeus is thick walled, much sclerotised, and forms the greater part of the clypeus. It is formed of two walls—the dorsal thick chitinous and the ventral thin and membranous. The dorsal and the ventral surfaces of the postclypeus are glabrous. The anterolateral membranous projection from the ventral side of the postclypeus is the clypealia. Because of the infolding of the cephalic margin of the postclypeus and the presence of the preclypeus on the anteroventral side, the clypealia also gets attached to the ventral surface of the preclypeus. Thus the clypealia becomes a

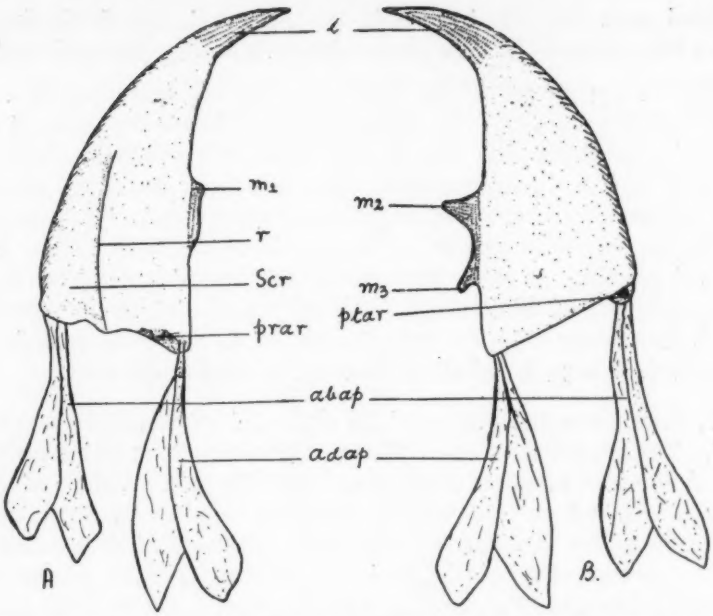
membranous projection of both the preclypeus and the postclypeus. Stickney (1923) has mentioned that the clypealia is a caudolateral projection of the postclypeus only. Ventrolaterally the clypealia bears a small crescentic socket, the precoila, for the articulation of the preartis of the mandible. The precoilæ are chitinous.

The labrum is a large, thick, chitinous and rectangular sclerite movably articulated to the preclypeus by a clypeolabral suture. Its dorsal surface is pubescent. The labrum is composed of two closely apposed walls—a dorsal thick and a ventral membranous one. Except on the caudal margin the labrum bears three marginal ridges on its periphery.

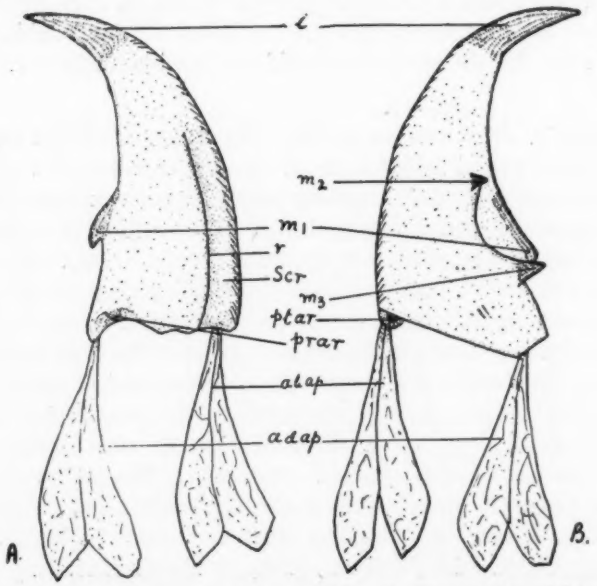
The ventral surface of the clypeolabrum forms the epipharyngeal wall of the preoral cavity. The labroepipharyngeal wall which is granulate at places, bears a flap-like structure in its middle forming the epipharynx proper. The epipharynx ends proximally near the oral aperture. The epipharyngeal wall beyond the base of the epipharynx is thin, membranous and smooth. It bears a shallow depression on the dorsal side for the epipharynx. On its caudal margin the labroepipharyngeal wall is supported by a thick semicircular bar to which are attached two short and broad epipharyngeal tendons. From the cephalolateral ends of the labral epipharynx to the base of the epipharynx run two longitudinal grooves, the labroepipharyngeal grooves. On the inner surface of the labroepipharyngeal wall opposite the grooves are two distinct longitudinal ridges.

(*b*) *Mandibles.*—The mandibles, situated laterad to the labium with the labrum hanging in between them, are directed forwards and inwards. Both the mandibles are large, stout, strongly cuticularised and project considerably in front of the head. Each mandible which is wide at the base and pointed at the apex has a rounded outer margin and a cutting inner margin. The cutting margin of each mandible bears cuticularised teeth which are distinguishable into upper incisors and lower molars. The number of incisors and molars in both the mandibles is the same, but their arrangement is different.

The cutting margin of the left mandible comprises of a single incisor at the distal end and three molars near the base. Between the first incisor and the first molar the mesial margin is sharp and straight without any indication of a tooth. This portion of the mandible is used as a cutting blade. The incisor is very long, curved inwards and pointed at the tip. The first molar, in the form of an edge of a glass plate, is sharp and raised upwards at the anterior end. The second and third molars are visible in the ventral view only. The second molar is long, straight, directed outwards, and bears



TEXT-FIG. 4. A. Dorsal view of the left mandible. B. Ventral view of the left mandible.



TEXT-FIG. 5. A. Dorsal view of the right mandible. B. Ventral view of the right mandible.

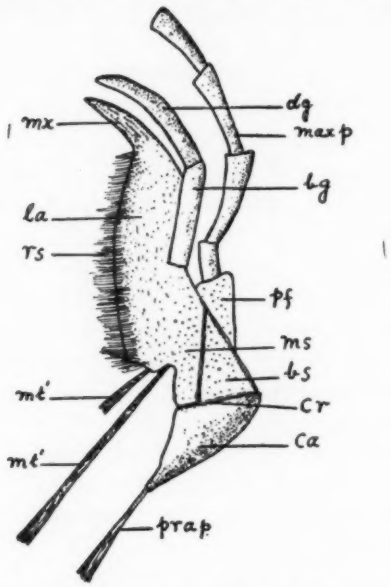
a pointed cusp. The third molar is short, directed towards the base and bears a blunt cusp. The three molars form a sort of a tricuspid tooth.

The cutting margin of the right mandible also comprises of a single incisor and three molars. The incisor is long, cuticularised, pointed at the apex and directed inwards. The incisors of the two mandibles overlap each other and work as a pair of scissors. The first molar is like the first molar of the left mandible but raised only at its posterior end. The second and third molars are visible only in the ventral view. The second molar is very short and pointed. As in the left mandible, the second molar is not situated between the first and third molars, but it takes a position on the ventral side towards the anterior end. Just near the base of the first molar runs the third molar which is large, broad at the base and pointed at the apex.

All the incisors and molars of the right and left mandibles are monocuspid. The mandibles form an efficient organ for seizing and grasping the prey. As in other members of the group Carabidæ each mandible of *A. sexguttata* is hollowed out and bears a prominent ridge on its dorsal surface. It is known as the scrobe (Andrewes, 1929). Each mandible has two articulatory processes—the anterior and the posterior articular processes—on its proximal margin. The anterior articular process, the preartis, fits into the precoila. The posterior articular process, the postartis, is in the form of a large rounded condyle which fits into the postcoila. To the base of each mandible are attached two very large apodemes, the inner adductor and the outer abductor, for the insertion of adductor and abductor muscles respectively.

(*c*) *Maxillæ*.—The maxillæ lie just behind the mandibles on either side of the mesially placed labium. Each maxilla consists of a cardo, stipes, lacinia, galea, palpifer, and maxillary palpus and articulates with the paracoila by means of a basal articular condyle of the cardo which also serves for the attachment of the promoter apodeme (Misra, 1945). The triangular cardo is separated from the stipes by a cardostipital ridge on the dorsal surface and a cardostipital suture on the ventral surface. It shows no division into a basi- or a disti-cardo, as is the case in most of the other beetles (Khatib, 1946). The stipes which is granulate on its dorsal surface forms the median portion of the maxilla. It is separated from the palpifer by a well developed longitudinal ridge arising from the lateral end of the cardospital ridge upto the caudolateral end of the galea. The stipes is composed typically of two parts—the basistipes and the mediostipes (Williams, 1938). The dististipes, a small membranous structure present in beetles like those of the family Cicindelidæ (Williams, 1938), is absent in *A. sexguttata*. The

basistipes is the triangular part separated from the mediostipes by a ridge running from the cardostipital ridge to the base of the galea. The mediostipes forms the upper larger part of the stipes and is not demarked from the lacinia by any suture or ridge on the dorsal surface, while on the ventral surface it is distinguishable from the lacinia by a distinct oblique suture.



TEXT-FIG. 6. Dorsal view of the left maxilla.

The mediostipes is irregular in outline and bears two movable lobes at its cephalic end, the inner lobe is the galea and the outer, the lacinia. The galea is divided into a basigalea and a distigalea. The basigalea and the distigalea are long and slender, resembling the maxillary palpus in shape. To the base of the galea on the ventral side is attached a short muscle tendon which gives support to the muscles that bring about the movement of the galea. The distigalea is devoid of any sensory hair or setæ. The lacinia is hook-shaped, chitinoid, and dentate with a convex mesial margin. The dorsal and ventral surfaces of the lacinia are setigerous. It bears a single mexadentes at its anterior end which is sharp, curved, and strongly chitinised. The function of the mexadentes is apparently that of holding and possibly of assisting in communicating the food to the mouth (Crampton, 1916). On the mesial margin, which is sharply flattened, the lacinia carries numerous long and short soft hair, the lacinarastra, directed towards the preoral cavity,

From the dorsal surface of the lacinia a long muscle tendon arises caudomesially which gives attachment to the muscles that control the movement of the lacinia. At the lateral side of the stipes and separated from it by a well-developed longitudinal ridge is another distinct sclerite, the palpifer, which bears a four segmented maxillary palpus. The first segment of the maxillary palpus is short and articulates with the palpifer while the rest three segments are long, cylindrical and broad at their distal ends. The anterior end of the last segment is beset with many small sensory setæ which have a gustatory function.

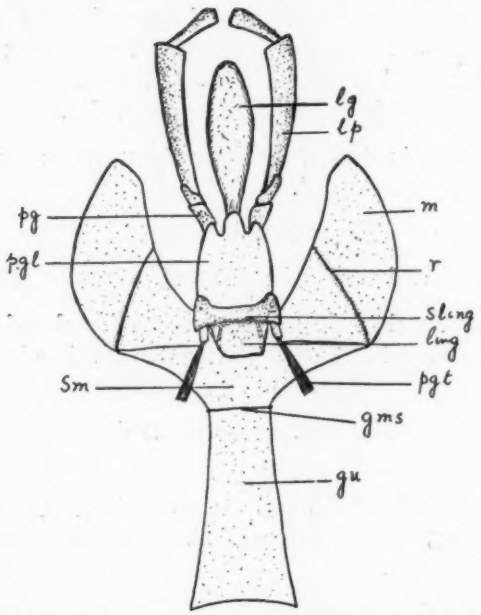
(*d*) *Labium.*—The labium in *A. sexguttata* is well developed and consists of a submentum, mentum, ligula, paraglossæ, palpigers and the labial palpi. The submentum is a small thick, much chitinous structure produced into two narrow lateral arms. Its dorsal surface is smooth. Anteriorly the submentum is separated from the mentum by a well-developed mental ridge and the dorsal and a mental suture on the ventral side. The posterior boundary of the submentum is marked off from the gula by a distinct gulomental suture. The posterior limit of the submentum is further confirmed by the presence of the metatentorinæ at each end of the gulomental suture (Snodgrass, 1935). Crampton's (1924) criterion of judging the posterior boundary of the submentum by means of a line drawn from one basal articulation of the cardo to the other also applies here for its satisfactory determination as both the paracoilæ and the metatentorinæ lie near the lateral ends of the submentum.

The thick-walled, strongly chitinoid, mentum comprises of two large leaf-like lobes separated from each other in front by a considerable gap and united behind. In the gap lie the various parts of the labium except the submentum. On the ventral side of each lobe an oblique ridge runs from its caudolateral end to its midmesial margin. The dorsal and ventral surfaces of the mentum are glabrous. As in most of the Coleoptera, a narrow membranous region, the mental membrane, is distinguishable between the mentum and the palpigers in *A. sexguttata.* Williams (1938) has mentioned that in some Coleoptera the mental membrane becomes confluent with the mentum.

From the anterolateral margin of the mental membrane on either side arise the two palpigers which are long, well developed and occupy a slightly oblique position ventrad to the hypopharynx and the paraglossæ. Each palpiger is granulate dorsally and bears a three-segmented labial palpus, the first segment of which is short. The second segment is long, broad, and cylindrical while the third is also cylindrical but narrow and short. The distal end of the third segment is membranous and sensory.

The ligula formed by union of the glossæ is very long, spatuliform and chitinous. Its dorsal surface is partly granulate. The paraglossæ are short, membranous and attached to the base of the ligula. The paraglossæ unite with each other by their mesial margin to form a short rectangular base for the attachment of the ligula. The anterolateral ends of the paraglossæ are pointed and separated from the anteromesial ends by a short narrow gap.

(*e*) *Hypopharynx.*—The hypopharynx in *A. sexguttata* is feebly developed and lies adnate to the posterior wall of the labium. Dorsally it is surrounded by the labrum and laterally by the maxillæ and mandibles. The hypopharynx is distinguishable into two membranous pad-like anterolateral lobes, the superlinguae (Folsom, 1900), which unite with each other mesially, and a caudal part, the lingua. The lingua, directed towards the oral aperture, is



TEXT-FIG. 7. Ventral view of the labium with hypopharynx.

beset with many short and pointed hair on the middorsal surface. The lingua and the superlinguæ are separated from each other by a shallow groove on the dorsal side and a corresponding ridge on the ventral side. The ridge gives attachment to two very short and narrow tendons, the hypopharyngeal tendons.

(*f*) *Tentorium.*—The endoskeleton or the tentorium is not well developed. It is very weak and non-chitinous. The posterior tentorial arms which are short and narrow open to the exterior through metatentorinæ at the ends of the gulomental suture. The anterior tentorial arms are longer than the posterior ones and broaden out distally to open on the dorsal wall of the head by means of linear furrows, the pretentorinæ. No œsotendons are given out from the anterior tentorial arms. The dorsal arms of the tentorium are also not developed.

V. SUMMARY

The present paper gives a detailed account of the morphology of the head capsule and mouth parts of the Indian Anthiini represented by only a single species *Anthia sexguttata* Ho. The following features are noteworthy.

1. The epicranial stem is absent and the epicranial arms lie in an invagination which is a compound structure representing the epistomal (frontoclypeal) suture and the entire frons. The invagination also forms the anterior and the posterior boundary of the vertex and the clypeus respectively.

2. Due to the reduction of the frons the vertex is much increased in extent and a clypofrons is formed.

3. The antennæ are kataceratous.

4. The occiput and the occipital sutures are well developed.

5. The cephalic end of the post-clypeus is infolded and carries the preclypeus on the ventral side. The clypealia is attached to both the pre- and postclypeus.

6. The epipharynx lies in a membranous depression strengthened by a semicircular ridge.

7. The number of incisors and molars in both the mandibles is the same but their arrangement is different. A scrobe is present on the dorsal surface of each mandible.

8. In maxilla a dististipes is absent.

9. The prognathous mouth parts comprise large maxillæ, mandibles and labium to form an efficient seizing orɜan.

10. The hypopharynx is feebly developed and adnate to the posterior wall of the labium.

11. The tentorium is weak and non-chitinous.

VI. Acknowledgements

We express our gratitude to Sri. Bhim Sen, Principal, Government College, Ajmer, for providing the research facilities.

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VIII. Abbreviations

ab ap	.. abductor apodeme	*cr*	.. cardostipital ridge
ad ap	.. adductor apodeme	*cs*	.. cardostipital suture
anf	.. antafossa	*dg*	.. distigalea
ant	.. antenna	*ep*	.. epipharynx
bg	.. basigalea	*ept*	.. epipharyngeal tendon
bl	.. bulb of the antenna	*fl*	.. flagellum
bs	.. basistipes	*fpge*	.. flat area of the postgena
ca	.. cardo	*frr*	.. frontal ridge
ce	.. compound eye	*ge*	.. gena
cl	.. clypeus	*gms*	.. gulomental suture
clf	.. clypofrons	*gs*	.. gular suture
clls	.. clypeolabral suture	*gu*	.. gula
clm	.. clypeal membrane	*i*	.. incisor
clp	.. clypealia	*inv*	.. invagination

l .. labrum
la .. lacinia
lepg .. labro-epipharyngeal groove
ling .. lingua
lg .. ligula
lp .. labial palpus
m .. mentum
m_1 .. first molar
m_2 .. second molar
m_3 .. third molar
maxp .. maxillary palpus
md .. mandible
mm .. mental membrane
mr .. marginal ridge
mr' .. mental ridge
ms .. mediostipes
mt .. metatentorina
mt' .. muscle tendon
mx .. mexadentes
oc .. occiput
ocs .. occipital suture
of .. occipital foramen
ol .. oculata
pd .. pedicel
pf .. palpifer
pg .. palpiger
pge .. postgena
pger .. postgenal ridge
pgl .. paraglossa
pgt .. palpiger tendon
pn .. pretentorina
poc .. postocciput
pocl .. postclypeus
pocs .. postoccipital suture
prap .. promotor apodeme
prar .. preartis
prc .. precoila
prcl .. preclypeus
ptar .. postartis
ptc .. postcoila
r .. ridge
rs .. lacinarastra
sc .. scape
scb .. semicircular ba
scr .. scrobe
sling .. superlingua
sm .. submentum
st .. stipes
v .. vertex

A CONTRIBUTION TO THE EMBRYOLOGY OF *THERIOPHONUM MINUTUM* ENGL.

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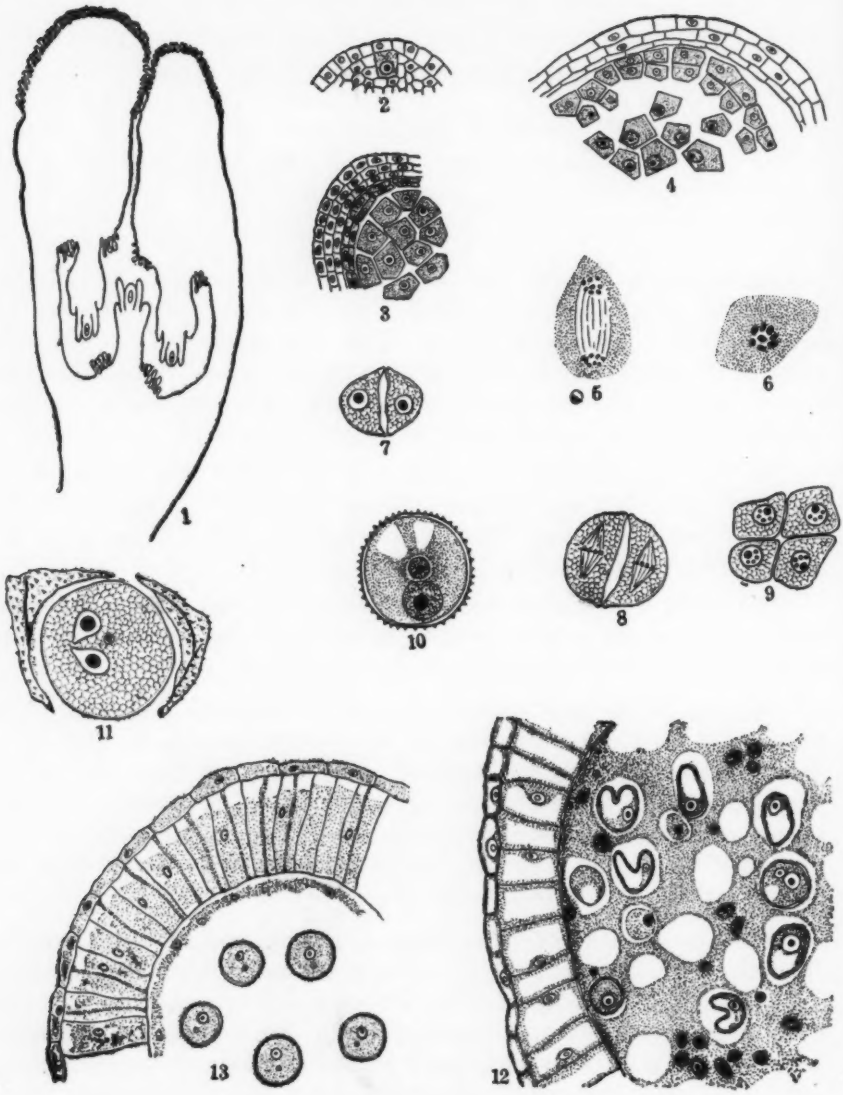
The embryological literature on the family Araceæ has been recently briefly reviewed by Maheshwari and Khanna (1956), in their investigation of the embryology of *Arisæma wallichianum*. There still appear to be considerable points that need clarification, among which the endosperm is one. The present study has been taken up in order to add more data in light of current understanding of the embryological features of the family.

Theriophonum minutum is monœcious. Male and female flowers are devoid of perianth parts, and are arranged acropetally on spikes enclosed within spathes. Each male flower has two sessile anthers; the connective is protruded slightly beyond the anthers.

The ovary is unilocular and has a short style and a hairy subsessile stigma. The ovules, which are four to six in number, are orthotropous. Both erect and pendulous ovules are present (Fig. 1).

Material of the plant was collected from Vandalur, 18 miles south of Madras and fixed in formalin-acetic-alcohol or in Craf solution. The plants grow in abundance along the bunds of paddy fields, and flower during September-October by which time the vegetative phase of the plant will be over. Customary methods of dehydration and embedding were followed. Sections were cut 5–10 microns thick and stained in Heidenhain's Hæmatoxylin and counter-stained in erythrosin or fast green.

Microsporogenesis and pollen grain.—The archesporium is differentiated very early in the hypodermal layer of the young anther primordium and consists of a single vertical file of cells. The cells are conspicuous by their relatively large size and prominent nuclei (Fig. 2). The archesporial cells divide periclinally to give rise to the primary parietal cell and primary sporogenous cell. The former by repeated anticlinal and periclinal divisions gives rise to three layers of cells (Fig. 4). The outermost of these, that is the one next to the epidermis, develops into endothecium at maturity. It must be mentioned, however, that the fibrous thickenings on the constituent cells are rather poorly developed although the cells invariably exhibit conspicuous radial



FIGS. 1–13

dimensions (Fig. 13). As in *Arisæma wallichianum* (Maheshwari and Khanna, 1956) the cells of this layer in the neighbourhood of the place of dehiscence of the anther are wholly unthickened in the present material also.

The innermost of the layers given off by the primary parietal cell differentiates into the tapetum. It is observed that many cells of this layer undergo a periclinal division to form two layers of cells (Fig. 3). The tapetal cells remain uninucleate throughout. At about the time when the microspores are being formed, the cells of the tapetum lose lateral contact with one another, the limiting wall breaks down and cytomixis occurs. The *en masse* protoplasts now begin to expand centripetally thereby ensheathing the developing microspores. Thus a typical periplasmodium becomes organized (Fig. 12). The presence of a periplasmodium is also characteristic of other araceous plants like *Dieffenbachia seguine* (Campbell, 1900), *Symplocarpus fœtidus*, *Peltandra undulata* (Duggar, 1900), *Aglaonema versicolor* (Gow, 1908 *b*), *Peltandra virginica* (Goldberg, 1941), *Typhonium trilobatum* (Banerji, 1947) and *Arisæma wallichianum* (Maheshwari and Khanna, 1956). In *Theriophonum minutum*, the periplasmodium becomes confined as a peripheral layer in the locule towards maturity, that is, when the pollen grains are two celled (Fig. 13).

Hand in hand with the division of the primary parietal cell, the primary sporogenous cell also undergoes repeated divisions in diverse planes so as to increase in bulk (Fig. 3). The resulting cells function as the microspore mother cells. The spore mother cells undergo the meiotic divisions in a successive manner (Figs. 5–9). During this reduction division it is observed that the haploid number of chromosomes is eight (Fig. 6). The nucleus of the microspore moves towards one side of the cell and cuts off a small generative cell, which after a time shifts its position so as to lie within the cytoplasm of the vegetative cell (Fig. 10). This two-celled condition of the pollen grain represents the shedding stage. The exine is relatively thin and shows tiny spinescent sculpturing (Fig. 10). It may also be noted that the epidermis of the anther wall does not become disoraganized at maturity (Fig. 13).

Megasporogenesis and embryo sac.—During the development of the ovule the inner integument originates earlier from the base of the nucellus (Fig. 14), and when this structure has grown to the height of the nucellus, the outer integument makes its appearance (Fig. 1). As has been reported in the other genera of the family, the initial growth of the inner integument is relatively rapid, but during later ontogeny the outer integument overgrows the inner, excepting in *Aglaonema versicolor* (Gow, 1908 *b*) where the two integuments appear to be more or less of the same height. For the most part, the inner integument is two cell layers in thickness, excepting at the micropylar end where it is three cell layers thick (Fig. 28). The outer integument, on the other hand, is uniformly made of six cell layers in thickness (before fertilization). The inner layer of the inner integument organizes,

rather weakly though, into an endothelium in which the constituent cells possess slightly larger radial dimensions.

The ovule is tenuinucellate. An archesporial cell differentiates in the sub-epidermal layer (Fig. 14). No parietal cell is formed by the division of the archesporial cell. On the other hand, the nucellar epidermal cells towards the apex and sides of the ovule undergo a division in the periclinal plane (Fig. 15). Thus, at the time of division of the archesporial cell—which directly functions as the megaspore mother cell—there will be two nucellar cell layers present (Fig. 15–19) until the organization of the young gametophyte (Fig. 20). Both the cell layers of the nucellus towards the sides of the gametophyte disintegrate at the time of formation of the four-nucleate embryo sac, while the concerned layers toward the apex persist in the form of a cap. This situation causes the sides of the embryo sac to come in direct contact with the weakly developed endothelium. The cells of the nucellar

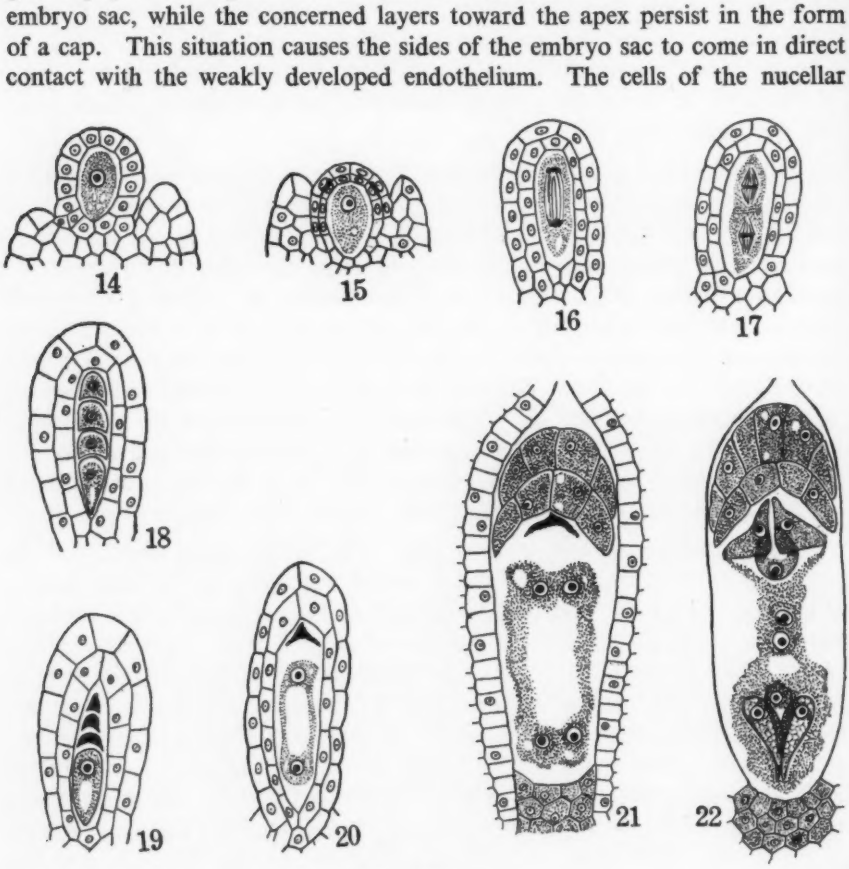


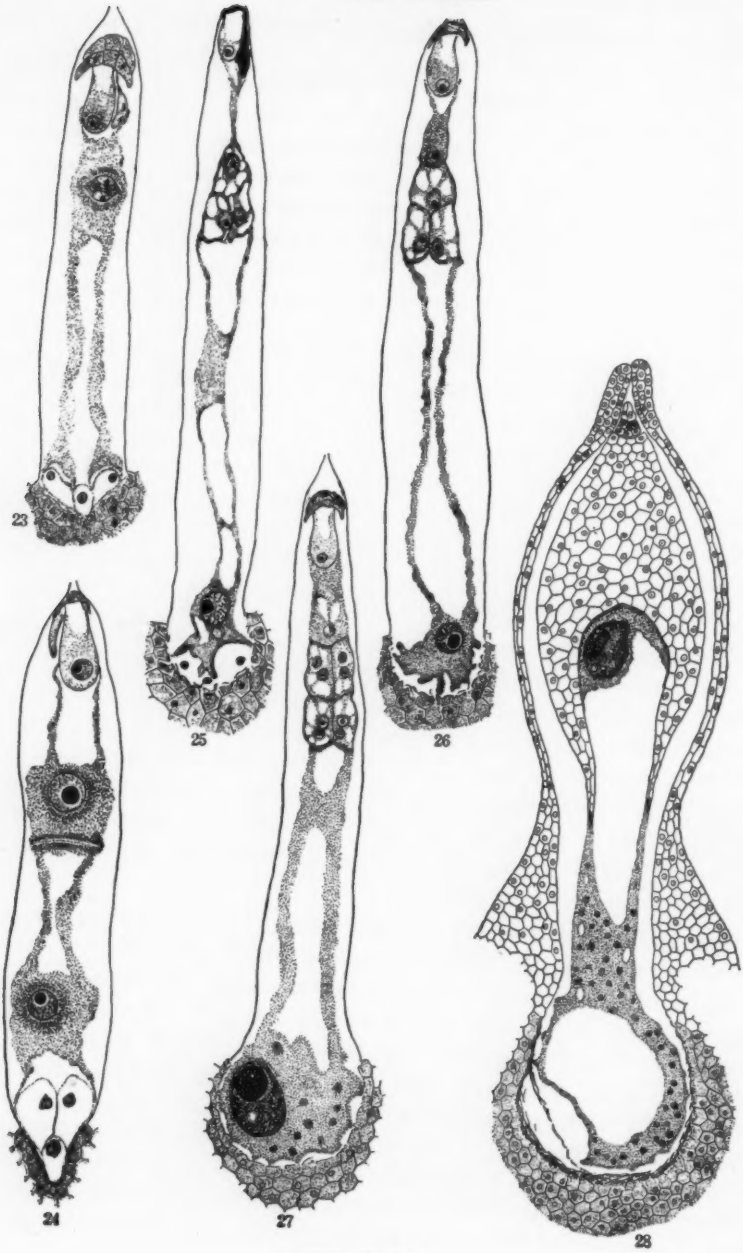
FIG. 14–22

cap become somewhat hypertrophied and exhibit relatively denser protoplasts (Fig. 21). The nucellar remains disintegrate and disappear during the early stages in the development of the endosperm (Fig. 24). Such a phenomenon has also been reported in many other representatives of the family thus far investigated, *e.g. Lysichiton* (Campbell, 1899), *Dieffenbachia seguine* (Campbell, 1900), *Aglaonema commutatum*, *Spathicarpa sagittæfolia* (Campbell, 1903), *Anthurium violaceum* var. *leucocarpum* (Campbell, 1905), *Arisæma triphyllum* (Gow, 1908 *a*), *Typhonodorum lindleyanum* (Boodle and Hill, 1929), *Peltandra virginica* (Goldberg, 1941), *Typhonium trilobatum* (Banerji, 1947) and *Arisæma wallichianum* (Maheshwari and Khanna, 1956).

The megaspore mother cell, after meiotic divisions, gives rise to a linear tetrad of megaspores (Figs. 16–18). The chalazal megaspore by three successive divisions of its nucleus organizes into the mature embryo sac (Figs. 19–22). The Polygonum type of embryo sac occurs in other members of the family also. In *Theriophonum minutum* the polar nuclei fuse at the equator of the embryo sac and the secondary embryo sac nucleus migrates towards the egg apparatus and lies *in situ* at the time of fertilization. The antipodal cells occupy the normal position in the embryo sac and persist until the initial stages of post-fertilization development (Fig. 24).

After the transference of pollen on to the stigmatic surface, the generative cell of the pollen grain divides to form two sperm cells. The sperm cells appear very conspicuous not only by their size but also by the sharply defined translucent areas surrounding the nuclei (Fig. 11). With further differentiation, the non-nucleolar part of the vegetative nucleus appears to shrink, ultimately leaving the nucleolus alone to persist for some more time. The actual formation of the pollen tube takes place only after the above-mentioned changes are brought about. The entry of the pollen tube is porogamous and double fertilization is accomplished.

Post-fertilization development.—The primary endosperm nucleus divides at a place nearer to the zygote (Fig. 23). A phragmoplast is organized during the division and after the daughter nuclei are formed, cytokinesis takes place, so that a relatively smaller micropylar and a larger chalazal chamber are formed (Fig. 24). The next cell generation in the endosperm takes place in the micropylar chamber; the ensuing wall is generally in a plane parallel to the one at the previous division. At this stage, it may be recalled that the endosperm consists of a micropylar chamber followed by a two-celled tier and a large chalazal chamber (Fig. 25). The micropylar chamber now divides by a transverse wall (Fig. 26) and the daughter cell adjacent to the two-celled tier divides by two vertical walls placed at right angles



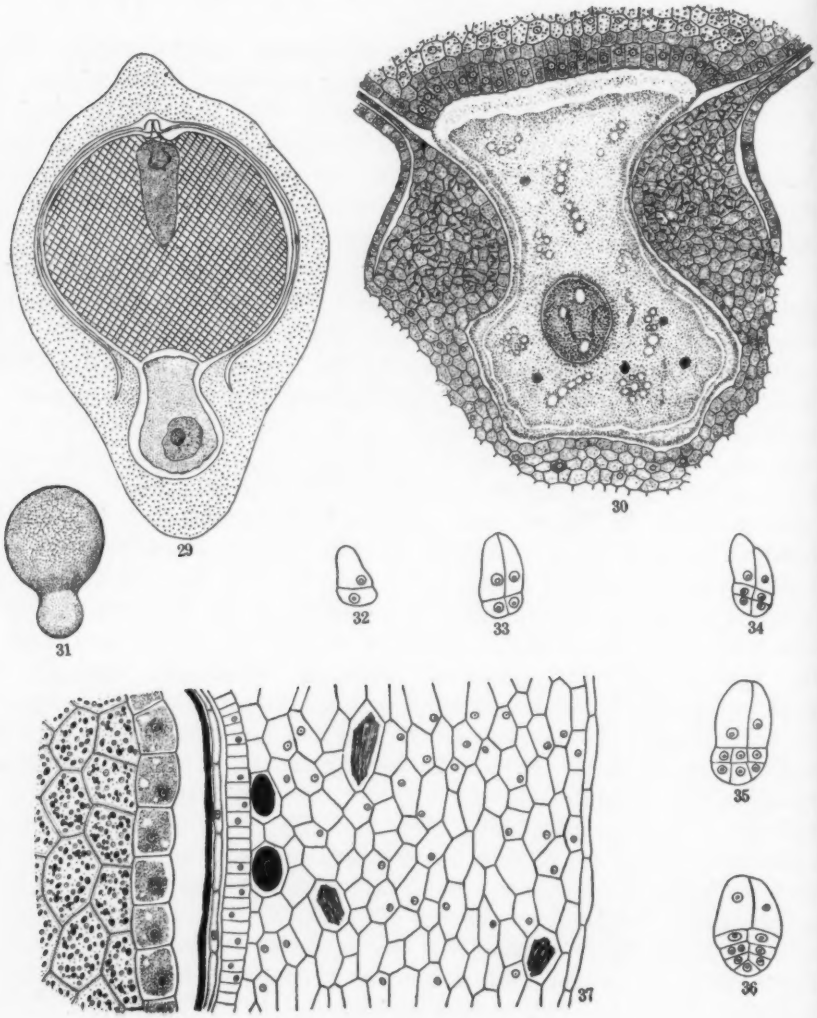
FIGS. 23–28

to each other. The lower tier also undergoes vertical divisions. Thus, two tiers of four cells each come to be organized (Fig. 27). The micropylar cell which is still relatively large undergoes repeated segmentation and the derivative cells assume the same morphologic form as of the cells or derivatives of the cellular part (Fig. 28). Thus, the entire cellular part of the endosperm is built by the micropylar chamber in which the first three or four cell generations conform to a pattern.

A central tier of four cells between the chalazal chamber and the micropylar cell has also been reported in *Arisæma wallichianum* (Maheshwari and Khanna, 1956). *Typhonium trilobatum* (Banerji, 1947) also appears to exhibit a similar stage in so far as the first two divisions of the primary endosperm nucleus are concerned; further stages are however lacking except for a statement that the " cells of the lower tier divide longitudinally while those of the upper might divide several times transversely".

The cellular endosperm increases in bulk at a fast rate so much so that the chalazal chamber appears rather minute (Fig. 29). From the two-celled stage of endosperm, the chalazal chamber begins to function as a true haustorium (Figs. 25–28). While it is true that the haustorium does not markedly invade the neighbouring tissues (excepting for a slight chalazal-ward extension),the cell undergoes over-all increase in surface area, and it may be pointed out that in dissections of mature seeds the haustorium is visible to the naked eye as a short peg popping out from the cellular mass of endosperm (Fig. 31). The nucleus of the chalazal haustorium undergoes conspicuous hypertrophy although its shape is not subjected to any bizarre distortion. The cell possesses vast amount of cytoplasm at maturity (Fig. 30), and the contents attain a viscous or almost rubber-like consistency (as seen in dissections). The wall of the haustorium, particularly towards the bottom and sides of the chalazal extremity, becomes excessively thickened; optically the thickened part of the wall does not present a lamellate structure, but appears to have become more or less amorphous and gelatinized (Fig. 30). The endosperm cells that neighbour the upper extremity of the haustorium are conspicuously elongate vertically and the superficial layer of the cellular endosperm elongate periclinally (Fig. 30). These superficial cells of the endosperm, irrespective of their topographical location, fail to store aleurone particles unlike the remaining cells of the endosperm (Fig. 37). An *ab initio* cellular endosperm has been reported in eleven out of the twenty-four species of Araceæ thus far investigated; in the remaining species the endosperm is either nuclear or helobial (for details *see* Maheshwari and Khanna, 1956). Amongst the species with cellular endosperm, the presence of a distinct chalazal haustorium is known only for *Peltandra virginica* (Goldberg, 1941),

Typhonium trilobatum (Banerji, 1947), *Arisæma wallichianum* (Maheshwari and Khanna, 1956) and *Theriophonum minutum* (present investigation). It must be admitted that much of the earlier work dealing with the embryology of Araceæ is either too brief or casual or is rather uncritical. Therefore, a lot more of careful work on a wider variety of araceous taxa is very



FIGS. 29–37

desirable in addition to a reinvestigation of many of the genera and species. Until such a time when reliable data becomes available, our knowledge of the embryological features of the family with particular reference to endosperm is bound to remain unclear.

The first division of the zygote is noticed only when the micropylar part of endosperm consists of a few cells (Fig. 32). The division of the zygote is by the formation of a transverse wall. Both the basal and terminal cells divide vertically (Fig. 33). The basal tier soon elongates conspicuously. The continued divisions in the terminal tier result in the formation of the embryonal mass (Figs. 34–36). The basal tier does not undergo further divisions and persists as a weakly developed supensor. The mature embryo is vertically elongate and cylindrical. It occupies a relatively small volume in relation to the endosperm (Fig. 29).

During post-fertilization development, the inner layer of the inner integument becomes filled with tanniferous contents, while the outer one persists. The outer integument increases in thickness. The cells, however, remain mostly parenchymatous, the cells of the innermost layer presenting more or less prismatic appearance. Some of the cells of this integument contain raphides and few others tanniferous compounds (Fig. 37).

Summary

During the development of the anther in *Theriophonum minutum* the uninucleate tapetum organizes a typical periplasmodium. The microspores undergo reduction in a successive manner. The pollen grains are shed at the two-celled stage.

The ovary is unilocular with four to six ovules. The ovules are bitegmic and tenuinucellate.

The archesporial cell directly functions as the megaspore mother cell. The nucellar epidermis towards the apex of the ovule undergoes a periclinal division and this part of the nucellus persists even during early post-fertilization development. The development of the embryo sac conforms to the Polygonum type.

Endosperm is cellular. The chalazal cell of the endosperm after the first division of the primary endosperm nucleus develops into a conspicuous haustorium while the cellular endosperm is contributed by the micropylar cell. At maturity, the superficial layer of the cellular mass of endosperm remains morphologically distinct by their characteristic absence of stored food materials in contrast to the internal cells. The embryo occupies a relatively small volume in contrast to the endosperm.

The inner integument in the seed becomes reduced to a papery layer, the cells of which exhibit tanniferous inclusions. The outer integument grows considerably in thickness and remains wholly parenchymatous with scattered cells containing raphides.

ACKNOWLEDGEMENT

It is a privilege to express my sincere gratitude to Dr. B. G. L. Swamy, Chief Professor of Botany, for initiating me into research and for his constant guidance and helpful criticisms during the course of this investigation. My thanks are also due to the Government of India for the award of a Senior Scholarship during the tenure of which this work was completed.

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EXPLANATION OF FIGURES

FIGS. 1–13. Fig. 1. L.S. of the ovary showing the topography of erect and pendulous ovules, ×65. Fig. 2. Archesporial cell in the anther primordium, ×260. Fig. 3. Wall of young anther showing two-layered tapetum, ×260. Fig. 4. The same, a later stage, ×260. Figs. 5–9. The successive stages in the development of microspores. Figs. 5, 6, 7 and 9, ×640. Fig. 8. ×920. Fig. 6. Metaphase chromosomes of Division II, ×640. Fig. 10. Pollen grain showing generative and vegetative cells, ×260. Fig. 11. Pollen grain separated from stigma

showing two sperm cells and shrunken vegetative nucleus, ×640. Fig. 12. T.S. of anther to show periplasmodial behaviour of the tapetum and the developing endothecium, ×260. Fig. 13. Mature anther showing the peripherally placed periplasmodial remains, weakly developed endothecium and persistent epidermis, ×260.

FIGS. 14–22. Fig. 14. Ovule primordium showing archesporium and initiation of inner integument, ×350. Fig. 15. Two-layered nucellar epidermis, ×350. Figs. 16–18. Divisions of the archesporial cell to form the linear tetrad, all Figs, ×350. Fig. 19. Germinating megaspore, ×350. Fig. 20. Two-nucleate embryo sac, ×350. Fig. 21. Four-nucleate embryo sac. The nucellar cap is seen at the micropylar end, ×350. Fig. 22. Mature organized embryo sac, ×350.

FIGS. 23–28. Fig. 23. The division of the primary endosperm nucleus. The persistent antipodals may also be seen, ×190. Fig. 24. Micropylar and chalazal chambers of the endosperm, ×280. Figs. 25–27. Successive stages in the development of endosperm, all Figs, ×190. Figs. 28. Mature endosperm with the chalazal haustorium, ×50.

FIGS. 29–37. Fig. 29. L.S. of mature seed showing the relative proportions of endosperm and embryo, ×15. Fig. 30. Chalazal haustorium showing dense nature of the cytoplasm and the hypertrophied nucleus, ×60. Fig. 31. Diagrammatic sketch of the endosperm mass drawn from dissection. Figs. 32–36. Stages in the development of the embryo, all Figs., ×215. Fig. 37. L.S. of seed showing a part of the seed coat and endosperm, ×215.

STUDIES ON SOME PHYSIOLOGICAL ASPECTS OF DIGESTION IN THREE SPECIES OF ELASMOBRANCHS

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A. Introduction

In fishes, although considerable work has been done on the anatomy and histology of the alimentary canal, very little is known of the physiology of digestion. The available literature has been reviewed by Yung (1889), Sullivan (1907), Yonge (1931), Vonk (1941) and Barrington (1942). Most of the work done so far is on the teleosts. The present investigation was taken up to obtain a comparative knowledge of digestion in elasmobranchs.

The work includes (i) the rate of digestion and (ii) the pH measurements of the alimentary canal. The following three species of elasmobranchs and two of teleosts were selected for the study:—

(*a*) *Elasmobranchs*

1. *Chilloscyllium griseum* (Mull and Henle).
2. *Dasyatis uarnak* (Forskal).
3. *Rhinobatus halavi* (Forskal).

(*b*) *Teleosts*

1. *Scatophagus argus* (Day).
2. *Therapon jarbua* (Forskal).

B. Material and Methods

Fishes for study were obtained in the living condition from Chowpatty, Mahaluxmi, Colaba and Bandra, not far from the Taraporevala Aquarium, Bombay, where this work was conducted. Live specimens, collected from the fish landing sites, were brought to the Aquarium and were immediately transferred to a conditioning tank, measuring 16′ length × 4′ breadth × 4′ height. Sea-water in this tank was kept well aerated. The fish were left in it for four to five days so as to acclimatise them to captivity conditions,

No food was given to the fish during this period. This treatment enabled us to select suitable fishes which would stand further treatment.

Fish from the conditioning tank were transferred to two storage tanks, each measuring 10′ length×4′ breadth ×4′ height. These tanks were provided with running sea-water. The fish were starved in these tanks for seven days so as to ensure that whatever food they had taken in before they were brought to the Aquarium was digested, leaving the alimentary canal completely empty.

After the starvation period, feeding experiments were conducted. Starved fish for this purpose were taken out from storage tanks and were transferred to a feeding tank, similar in dimensions to those of the storage tanks. Only one fish was taken out at a time and was fed on chopped pieces of prawns. If the fish took the food readily, it was then transferred to an experimental tank, measuring 6′ length×2′ breadth×2′ height. The fish was left in this tank for the period required under experiment. If, however, the fish did not take food readily, it was taken out and the food material was forcibly introduced into its stomach through the mouth with the help of a smooth glass rod. Those specimens which did not vomit the food and reacted well to this artificial type of feeding were also used for further investigation. The food was allowed to remain in the alimentary canals of all the species for varying periods of 8, 18, 24, 32, 48, 52 and 72 hours. The fish were then killed by pithing and the alimentary canals opened to observe the condition of food.

pH Measurements

pH measurements were taken to observe the rise and fall in acidity and alkalinity in elasmobranch fishes in starved condition and in those in which the food was allowed to remain for 24, 48 and 72 hours.

Fish were killed by pithing and this operation as well as the removal of the alimentary canal had to be carried out very swiftly and with great care in order to prevent mixing of the fluid from the stomach with that from the intestine and *vice versa*. The alimentary canal was ligatured at the œsophagus, pyloric constriction and at the cloaca. It was then cut open and kept in a paraffin dish. pH measurements were taken on Photovolt Model 125 pH meter.

The calomel electrode was placed at one end of the canal and by shifting the glass electrode at various places the pH readings were noted. The glass electrode was always rinsed in distilled water when kept at a fresh place. The pH meter was standardised every time the pH measurements of each alimentary canal were taken.

C. OBSERVATIONS

(*a*) *Rate of Digestion*

All the fishes were fed with prawns. The temperature of the water was kept more or less constant at 80° F. The condition of the food after specified hours is given in Table I.

(*b*) *Hydrogen-ion Concentration in the Alimentary Canal*

Chiloscyllium griseum

Fish No. I (Fig. 1 *a*).—A live specimen of *Chiloscyllium griseum* was kept without food for seven days in an aquarium tank containing clear sea-water. No food was found in the alimentary canal after the starvation period. pH values found are as follows:—

	pH
(*a*) At one place in the œsophagus	4·5
(*b*) At three different places on the left-hand side of the corpus	4·3, 4·5, 4·8
(*c*) At two different places on the right-hand side of the corpus	4·7, 4·8
(*d*) In the middle region of the corpus	4·2
(*e*) At three different places in the pylorus	4·8, 5·4, 5·6
(*f*) At three different places in the intestine ..	7·1, 7·2, 7·5

Fish No. II (Fig. 1 *b*).—A fish was fed with pieces of prawns and fish. pH measurements were taken after the food had been in the stomach for 24 hours.

	pH
(*a*) At one point in the œsophagus	5·0
(*b*) At three different places on the left-hand side of the corpus	3·9, 3·7, 4·5
(*c*) At three different places on the right-hand side of the corpus	3·9, 3·7, 4·0
(*d*) At three different places in the middle region of the corpus	3·5, 3·4, 3·3
(*e*) At three different places in the pylorus	5·0, 5·6, 5·7
(*f*) At three different places in the intestine ..	6·4, 7·2, 7·2

Fish No. III (Fig. 1 *c*).—The fish was fed with pieces of prawns and fish. pH measurements were taken after the food had been in the stomach for 48 hours.

	pH
(*a*) At one place in the œsophagus	4·2
(*b*) At two different places on the left-hand side of the corpus	4·1, 3·8
(*c*) At two different places on the right-hand side of the corpus	4·5, 4·6
(*d*) At three different places in the middle region of the corpus	3·6, 3·3, 3·4
(*e*) At three different places in the pylorus	5·0, 5·6, 6·0
(*f*) At two different places in the intestine	6·3, 7·2

Fish No. IV (Fig. 1 *d*).—The fish was fed with pieces of prawns and fish. pH measurements were taken after the food had been in the digestive canal for 72 hours.

	pH
(*a*) At one place in the œsophagus	5·5
(*b*) At three different places on the left-hand side of the corpus	5·2, 4·8, 5·2
(*c*) At three different places on the right-hand side of the corpus	5·2, 5·2, 5·4
(*d*) In the middle region of corpus	4·2
(*e*) At three different places in the pylorus ..	5·6, 5·5, 7·5,
(*f*) At three different places in the intestine ..	7·8, 7·7, 7·8

Dasyatis uarnak

Fish No. I (Fig. 2 *a*).—A live specimen of *Dasyatis uarnak* was kept in an experimental tank containing sea-water and starved for seven days.

	pH
(*a*) At one place in the œsophagus	4·5
(*b*) At two different places on the left-hand side of the corpus	4·7, 4·1
(*c*) At two different places on the right-hand side of the corpus	4·0, 3·8
(*d*) At one place in the pylorus	5·8
(*e*) At three different places in the intestine ..	5·8, 6·0, 6·1

TABLE I

Name of fish	8 hours	18 hours	24 hours	32 hours
Chiloscyllium griseum ..	Food in the corpus region. The prawns were acted upon by the gastric enzymes and were slightly reduced to pulpy condition.	Food in the pulpy condition in corpus. A little of food from the corpus had passed into the pylorus. No food had passed into the intestine.	Food in the anterior region of the intestine in a pulpy condition. Very little food in the corpus. No food in pylorus.	Food had passed into the anterior region of the intestine and reduced to pulpy form. A little of food in corpus. No food in pylorus.
Dasyatis uarnak ..	Food in the corpus region. The prawn had become soft by the action of enzymes.	Food in pulpy form in the corpus. A little of food had passed into the pylorus. No food had passed into the intestine.	Food had passed into the anterior region of the intestine and in a pulpy condition. A little of food still in corpus and pylorus.	Food found in the anterior intestine and reduced to pulpy form. A little of food in the corpus and pylorus.
Rhinobatus halavi ..	Food in corpus. The prawns were acted upon by gastric enzymes and were reduced in a pulpy form.	Food in pulpy condition in corpus. A portion of food had passed into the pylorus. No food had passed into the intestine.	Food had passed into the anterior region of the intestine and in a pulpy form. Very little food still in the corpus and pylorus.	Food had passed into the anterior region of the intestine. Very little food in the corpus and pylorus.
Scatophagus argus ..	Food in the corpus. The prawns were slightly acted upon by the enzymes.	Food in the corpus in a pulpy condition. No food had passed into the intestine.	Food in the corpus and seen in pulpy form. No food had passed into the intestine.	Food in pulpy condition in the corpus. No food had passed into the intestine.
Therapon jarbua ..	Food in the corpus. Prawns were slightly acted upon by the enzymes.	Food in the corpus. Prawns were sligetly acted upon by the enzymes.	Food in the corpus and in a pulpy form. No food had passed into the intestine.	Food in the corpus and in pulpy condition. No food had passed into the intestine.

Chiloscyllium griseum ..	Food in the pesterior region of the intestine and reduced to pulpy form. Very little food in the corpus. No food in the pylorus.	Food in the posterior region of the intestine and in pulpy condition. A little of food had passed into the rectum. No food in the pylorus. Very little food in the corpus.	Very little food in the posterior region of the intestine. A little of food still in corpus.	No food in the stomach or in the intestine.
Dasyatis uarnak ..	Food in the middle region of the intestine and reduced to pulpy form and a little of food in corpus.	Food in the posterior region of the intestine. Very little food in corpus.	Food in the posterior region of the intestine and in pulpy form. A little food in the corpus.	No food in stomach. Very little food in the posterior region of the intestine.
Rhinobatus halavi ..	Food in the middle region of the intestine and reduced to pulpy form. Very little food in the corpus.	Food in the posterior region of the intestine. Very little food in the corpus.	Food in the posterior region of the intestine and in pulpy form. Very little food in the corpus.	No food in stomach. Very little food in the intestine.
Scatophagus argus ..	Food in pulpy form in the corpus. Very little food had passed into the pylorus. No food had passed into the intestine.	Food in pulpy condition in the corpus. A little food had passed into the pylorus. No food had passed into the intestine.	Food had patsed into the pylorus. Little food in the corpus. No food had passed into the intestine.	Food had just entered the anterior region of the intestine. A little food in the corpus and pylorus.
Therapon jarbua ..	Food in the corpus in pulpy form. Little food had passed into the pylorus. No food had passed into the intestine.	Food in the corpus in pulpy form. A little food had passed into the pylorus. No food had passed into the intestine.	Food had passed into the pylorus. Part of food still in corpus. No food had passed into the intestine.	Food had reached the anterior region of the intestine. Very little food in corpus and pylorus.

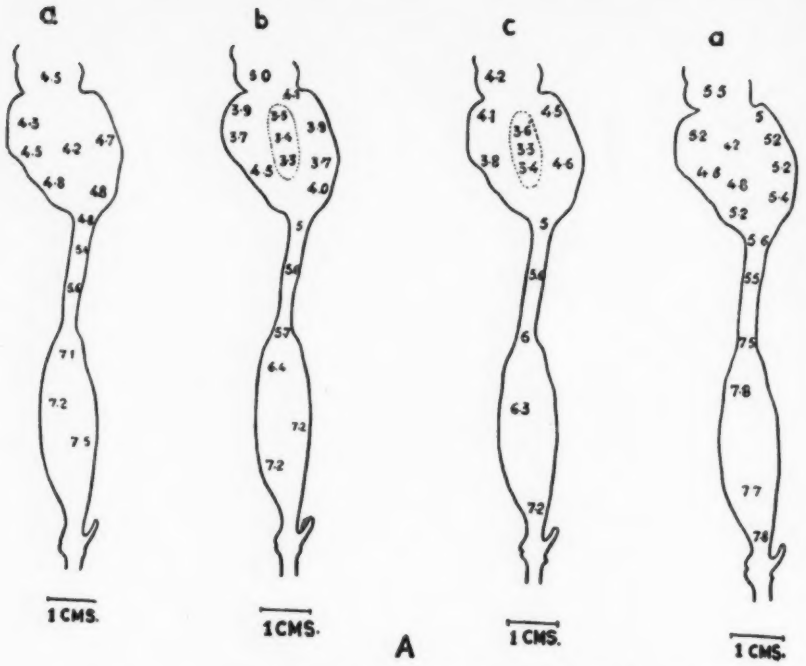

FIG. 1. pH measurements in the alimentary canal of *Chiloscyllium griseum.*

Fish No. II (Fig. 2 *b*).—The fish was fed with pieces of prawns and fish. pH measurements were taken after the food had been in the stomach for 24 hours.

	pH
(*a*) At one place in the œsophagus	5·1
(*b*) At one place in the left-hand side of the corpus ..	4·5
(*c*) At two places on the right-hand side of the corpus	4·2, 4·0
(*d*) At three different places in the middle region of the corpus	2·6, 2·8, 2·8
(*e*) In the pylorus	3·0
(*f*) At four different places in the intestine	4·2, 4·3, 6·1, 6·1

Fish No. III (Fig. 2 *c*).—The fish was fed with prawns and fish. pH readings were taken after the food had been in the stomach for 48 hours.

	pH
(*a*) At one place in the œsophagus	5·6
(*b*) At two different places on the left-hand side of the corpus	4·4, 4·4
(*c*) At two different places on the right-hand side of the corpus	4·3, 4·2
(*d*) At one place in the middle region of the corpus ..	3·9
(*e*) At one place in the pylorus	6·2
(*f*) At five different places in the intestine	6·4, 6·4, 7·2, 7·4, 7·6

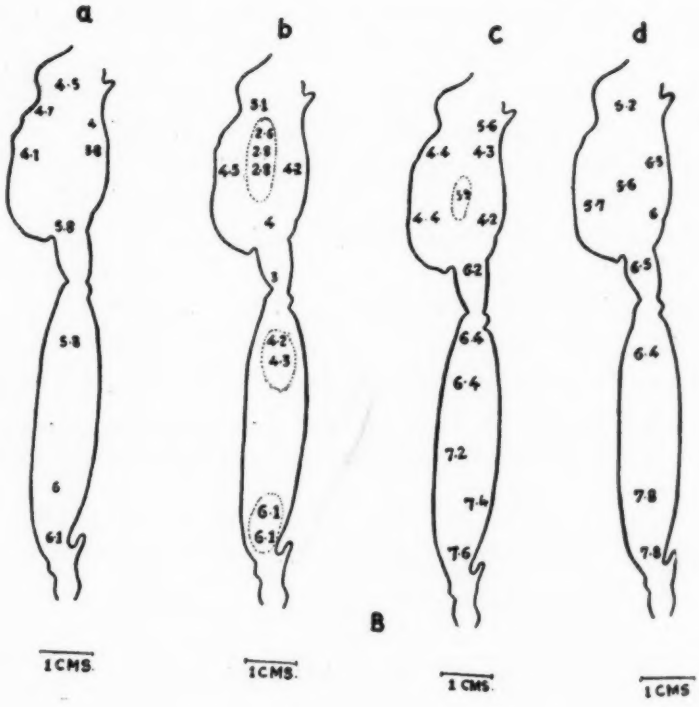

FIG. 2. pH measurements in the alimentary canal of *Dasyatis uarnak*.

Fish No. IV (Fig. 2 *d*).—The fish was fed with prawns and fish. pH measurements were taken after the food had been in the stomach for 72 hours.

	pH
(*a*) At one place in the œsophagus	5·2
(*b*) At one place on the left-hand side of the corpus	5·7
(*c*) At two different places on the right-hand side of the corpus	6·3, 6·0
(*d*) In the middle region of the corpus	5·6
(*e*) In the pylorus	6·5
(*f*) At three different places in the intestine ..	6·4, 7·8, 7·8

Rhinobatus halavi

Fish No. I (Fig. 3 *a*).—The fish was starved for seven days and pH readings were taken after the starvation period.

	pH
(*a*) At one place in the œsophagus	4·5
(*b*) At two different places on the left-hand side of the corpus	4·5, 4·2
(*c*) At four different places on the right-hand side of the corpus	3·4, 4·0, 3·8, 3·3
(*d*) In the middle region of the corpus	4·5
(*e*) In the pylorus	6·4
(*f*) At three different places in the intestine ..	6·8, 7·0, 7·2

Fish No. II (Fig. 3 *b*).—The fish was fed with prawns and fish. pH measurements were taken after the food had been in the stomach for 24 hours.

	pH
(*a*) At one place in the œsophagus	4·9
(*b*) On the left-hand side of the corpus	4·1
(*c*) On the right-hand side of the corpus	4·0
(*d*) At two different places in the middle region of the corpus	3·5, 4·5
(*e*) In the pylorus	5·6
(*f*) At three different places in the intestine ..	6·5, 6·9, 7·1

Fish No. III (Fig. 3 *c*).—The fish was fed with prawns and fish. pH measurements were taken after the food was in the stomach for 48 hours.

	pH
(*a*) At one place in the œsophagus	4·5
(*b*) At one place on the left-hand side of the corpus	4·2
(*c*) At one place on the right-hand side of the corpus	4·0
(*d*) In the middle region of the corpus	4·0
(*e*) In the pylorus	5·0
(*f*) At three different places in the intestine ..	7·0, 7·5, 7·1

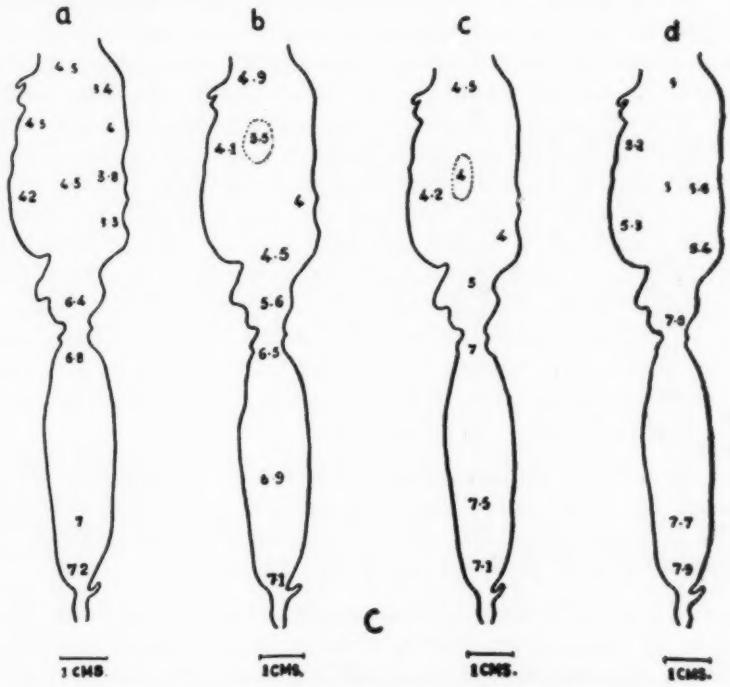

FIG. 3. pH measurements in the alimentary canal of *Rhinobatus halavi*.

Fish No. IV (Fig. 3 *d*).—The fish was fed with prawns and fish. pH measurements were taken after the food had been in the stomach for 72 hours.

	pH
(*a*) At one place in the œsophagus	5·0
(*b*) At two different places on the left-hand side of the corpus	5·2, 5·3

(*c*) At two different places on the right-hand side of the corpus	5·6, 5·4
(*e*) In the pylorus	7·0
(*f*) At two different places in the intestine ..	7·7, 7·9

D. DISCUSSION AND CONCLUSIONS

Rate of Digestion

Comparing the rate of digestion in the three species of elasmobranchs with that of the two species of teleosts, it was observed that 18 hours after feeding, the food contents in the three elasmobranchs were reduced to pulpy condition in the corpus. In the two teleostean species, the food contents after the same period of feeding were only partially digested in the corpus. After 24 hours of feeding, the gastric digestion was complete and the food had entered the intestine in the three elasmobranchs whereas in the teleosts the food was reduced to pulpy form and was seen lying still in the corpus region. After 32 hours of feeding, the pulpy food was seen in the anterior region of the intestine in the elsmobranchs, while the food remained in pulpy condition in the corpus of the teleosts. After 36 hours of feeding, the food was seen lying in the posterior region of the intestine in *Chiloscyllium griseum*. In *Dasyatis uarnak* and *Rhinobatus halavi* the food contents were seen in the middle region of the intestine. In the teleostean forms, very little of the pulpy food had entered in the pylorus.

After 48 hours of feeding, the food contents in *Chiloscyllium griseum* had reached the rectal region, whereas in *Dasyatis uarnak* and *Rhinobatus halavi*, the food had entered into the posterior regions of the intestines. In the teleostean forms the food was seen in the pylorus.

After 72 hours of feeding, food was neither seen in the stomach nor in the intestine of *Chiloscyllium griseum*, while in *Dasyatis uarnak* and *Rhinobatus halavi* a little portion of the food was still seen in the posterior regions of the intestines. In the teleostean forms the food in the pulpy form had reached the intestine.

It can be safely concluded from the aforesaid observations that, under the same conditions of feeding, the rate of digestion is faster in the elasmobranch forms than in the teleostean species. Among the three elasmobranch species, it was observed that the food contents get digested earlier in *Chiloscyllium griseum* than in the other two species, probably because *Chiloscyllium griseum* is the fastest moving species of the three elasmobranch

forms, thus demanding upon more energy to be liberated in the body and greater rate of metabolism.

H-ion Concentration

It is observed that the reactions in the œsophagus of the three species of elasmobranchs are acid. Yung (1899), Sullivan (1905), Herwerden (1908), Vonk (1929) and Beauvalet (1933) ascertained that hydrochloric acid and pepsin in fish are produced only in the corpus region of the alimentary canal. The acid reaction in the œsophagus would suggest that the acid secretion from the corpus enters the œsophagus. The flow of secretion from the corpus to the œsophagus is possible because the two are in communication, there being no separating valve.

The corpus of the fasting specimens of *Chiloscyllium griseum*, *Dasyatis uarnak* and *Rhinobatus halavi*, gave acid reaction. This would suggest that acid is secreted during the fasting period. Kewalramani (1953) observed that the reactions in the stomach of the fasting specimens of the two teleostean forms *Zoarces viviparus* and *Cottus scorpius* were neutral or alkaline but not acid. It was suggested that either no acid was secreted or acid which was secreted was neutralised by the gastric mucus, which is alkaline in reaction.

It can be concluded that, during the fasting period, the acid secretion in corpus region of elasmobranchs is of such high concentration that it does not get neutralised by gastric mucus to neutral or basic reaction as it happens in the teleostean species.

The pH measurements in the corpus region of the alimentary canals of the three species taken 24, 48, 72 hours after feeding indicate that the acid secretion is strongest after the food had been in the stomach for 24 hours. The acid concentration falls down, *i.e.*, the pH values rise in the corpus, after 48 and 72 hours of feeding. This can be explained from the observations made on the rate of digestion. It is observed that the digestion in the corpus was complete after 24 hours of feeding. So long as the food is present in the corpus, the gastric glands in this region pour out acid secretion of fairly high concentration. After this period when the food leaves the corpus and enters the intestine, the activity of gastric glands may be reduced thus lowering the acid concentration.

In all the specimens of *Chiloscyllium griseum*, *Dasyatis uarnak* and *Rhinobatus halavi*, it is found that pH values are generally lower in the corpus than in the pylorus. The average pH values observed in the corpus of *Chiloscyllium griseum* after fasting period, 24, 48 and 72 hours after introducing

food are 4·5, 3·6, 3·9 and 5·0 respectively; the average pH values in the pylorus under the same conditions are 4·9, 5·4, 5·8 and 6·2 respectively; the average pH values in the corpus region of the stomach of *Dasyatis uarnak* under the same conditions are 4·4, 3·8, 4·2 and 5·5 respectively, the pH values in the pylorus are 5·8, 5·2, 6·3 and 6·4 respectively; the pH values in *Rhinobatus halavi* under the same conditions in the corpus are 4·0, 3·8, 4·5 and 5·1 respectively, while in the pylorus are 6·4, 6·7, 6·9 and 7·0 respectively. These differences in pH values between the corpus and the pylorus indicate that the former is more acid than the latter. It seems likely, therefore, that the acid secretion takes place in the corpus, the pyloric being acidic only, because it receives acid secretion from the corpus. The two parts are in open communication in the stomach of the three species.

The secretion of the acid in the corpus is quite in accordance with the histological observations which we have made that the gastric glands are found in the corpus and not in the pylorus. Since the other histological features of the two regions of the stomach in the three species are similar, it seems likely that the acid in the corpus is secreted by the gastric glands. The pH values on the food substances lying in the stomach are lower than the pH values on the stomach wall.

In the anterior region of the intestine of *Chiloscyllium griseum*, *Dasyatis uarnak* and *Rhinobatus halavi*, pH measurements taken 24 hours after feeding, indicate that the reactions are slightly acid. But as the distal end of the intestine is approached the reactions become more and more alkaline. It is only in the case of *Dasyatis uarnak* that the reactions are acid, throughout the intestine. After 48 hours of feeding, the reactions are still slightly acid in the anterior region of the intestine. The middle and the proximal portions of the intestine are more alkaline than when the observations are made after 24 hours. After 72 hours of feeding, the entire intestine in all the three species is alkaline. The intestinal secretion is therefore alkaline in reactions and that the acid reaction in the anterior region of the intestine after the food had been in the stomach for 24 and 48 hours would suggest that much of the acid is secreted during the gastric digestion and that the acid from the stomach has passed into the intestine.

SUMMARY

The rate of digestion has been studied in three elasmobranchs and two teleosts by critical examination of food contents during fixed intervals after feeding. Among the three elasmobranchs, the rate of digestion is faster in *Chiloscyllium griseum* than in either *Dasyatis uarnak* or *Rhinobatus halavi*.

Comparing this with the teleostean species, *Scatophagus argus* and *Therapon jarbua*, it is observed that digestion is faster in elasmobranchs than in the teleosts, under similar conditions of feeding.

pH measurements were taken in the alimentary canals of the three species of elasmobranchs after starved conditions and after certain fixed intervals of feeding. The reactions in the stomachs of fasting fishes, 24, 48 and 72 hours after feeding, were acid. The acid secretion had the highest concentration after the food remained in the stomach for 24 hours. The concentration falls, *i.e.*, pH value rises in the corpus, after 48 and 72 hours.

The reactions in the anterior region of the intestine were acid after the food remained in the stomach for 24 and 48 hours. This seems to suggest that the acid secreted from the stomach entered the intestine. The entire intestine in all the three species was alkaline within 72 hours after feeding.

Acknowledgement

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EXAMINATION OF COMMERCIAL LEGUME INOCULANTS FOR QUALITY

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Introduction

In the year 1952, the New South Wales Department of Agriculture introduced the use of "peat-based" legume inoculants. The demand for these inoculants was so great that the Department could not handle the production and therefore handed over the business to private firms. At the moment three private firms in Australia are engaged in the manufacture of these inoculants which find a sale in all the States in Australia, besides being exported to New Zealand. As complaints of the general unsatisfactory nature of these inoculants began to accumulate, a scheme for testing the quality of the inoculants, prior to sale, was set on foot under the auspices of the University Department of Agriculture Laboratory Service (UDLAS). This paper deals with an account of the techniques used and results obtained from an examination of some representative samples of "peat-based" inoculants manufactured in Australia.

Material and Methods

"Peat-based" inoculants are examined in the laboratory for moisture, presumptive count of viable rhizobia per gm. of the inoculant, ability to nodulate the specific host and effectiveness in terms of plant benefits conferred on the host. As the main object of the paper is to describe the techniques followed in the study and discuss the factors which largely determine quality, the results of examination of only representative samples intended for inoculation of Clovers (*Trifolium* sp.) are given.

The fifteen inoculants studied were those manufactured by three different firms, five from each, *viz.*, (i) "Nodulaid" manufactured by Agricultural Laboratories, Pty. Ltd., N.S.W., Sydney, (ii) "Inoculo" manufactured by Inoculo Laboratories, Melbourne, Victoria, and (iii) those manufactured by Root Nodule Pty. Ltd., N.S.W., Sydney. The dates of expiry of utility of

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the inoculants were 27–8–1957, 30–9–1957 and 4–10–1957 respectively. In all these unsterilised peat was the carrier for rhizobia.

Experimental

(i) *Determination of moisture.*—The moisture content of the inoculant was determined by using L.K.B. Moisture Tester.† A sample of the inoculant was placed on the pan enough to lower the pointer to the zero line. The sample will then weigh 10 gm. The heater lamp is put in position above the pan and connected to the power supply. As the moisture is driven off by the heat of the lamp, the pan and the pointer rises on the moisture scale of 0–30%. For samples containing more than 30% moisture the zero point of the balance is depressed by placing a suitable weight on the pan when the moisture range between 0–100% is covered.

(ii) *Determination of nodulation and effectiveness.*—For this a seedling agar tube method was used. Surface sterilisation of the seed was carried out with alcohol and mercuric chloride on the lines described by Allen (1953). The seeds were then left in a sterile flask under sterile water for two hours. At the end of the period the seed was transferred to a petri plate containing sterile water agar of 1·5% strength. The seeds were spread out evenly in the plate with a sterile needle and the plate was incubated at 26° C. Sterile seedlings were ready in about 48 hours for transference to seedling agar tubes.

The nitrogen-free agar medium used for raising the plants in agar tubes was that described by Vincent and Waters (1953) and had the following percentage composition (W/V). $CaHPO_4$ 0·1; K_2HPO_4 0·02; $MgSO_4$ 7 H_2O 0·02; NaCl 0·02; $FeCl_3$ 0·01 and agar 0·8. The nitrate controls contained KNO_3 at 0·05%. The pH of the medium was adjusted to 6·5 with sodium hydroxide and 10 ml. of the medium was dispensed into each flat bottomed test-tube ($6'' \times 1\frac{1}{4}''$). The tubes were plugged with cotton and sterilized at 15 lb. pressure for 15 minutes. With a flamed forceps three germinated seedlings were transferred to each tube and sown into agar, using a " Sheperd's Crook " needle (a mounted needle with its tip bent in the form of inverted ' U ' and ' U ').

One gram of the inoculant was serially diluted with Ringer's solution to obtain a dilution of 10^8. One ml. of each dilution from 10^5 to 10^8 were added to duplicate tubes immediately after sowing the seed. The addition of a small pinch of the inoculant itself to duplicate tubes represented zero dilution. Against these inoculated nitrate-free experimental tubes two types

† L.K.B.-Produkter, Fabriksaktiebolag, P.O. Box 12035, Stockholm–12, Sweden.

of controls were provided, *viz.*, (*a*) nitrate control containing nitrate as KNO_3 0·05% in the agar medium and (*b*) uninoculated nitrate-free controls.

TABLE I

Results of experimental work on the different inoculants tested on red and subterranean clovers

Inoculant	Moisture per cent.	On moisture-free basis		Contaminants*	Effectiveness† per cent.†
		MPN 10^6	Viable count 10^6		
Nodulaid					
1	52	0·52	5·20	H	92·5
2	53	0·53	4·25	F	98·6
3	54	54·34	34·77	M	97·5
4	53	5·31	6·38	M	107·8
5	51	1·83	2·04	F	112·8
Inoculo					
6	53	53·19	2·12	H	127·6
7	54	0·54	15·22	F	112·8
8	53	5·31	14·89	H	113·5
9	53	1·91	23·41	F	98·7
10	52	0·52	3·75	F	114·6
Root nodule					
11	51	2·65	2·65	H	132·5
12	52	5·20	0·52	M	109·8
13	52	145·80	14·58	F	102·5
14	53	2·76	4·25	H	98·7
15	51	1·02	2·04	M	97·6

* H = High; M = Moderate; F = Few.

† Effectiveness = $(a-c/b-c)\times 100$, where a = weight of plants in 'test' plant tubes, b = weight of plants in nitrate control and c = weight of plants in uninoculated nitrate-free control. Samples 1–5 were tested on 3rd May 1957 and others on 4th May 1957.

The basal part of these tubes was wrapped in dark brown paper and the tubes kept in wooden racks were placed in a window box or under fluorescent light. The growth and nodulation were observed at regular intervals. After 8–10 weeks or when uninoculated controls showed extreme nitrogen deficiency symptoms the plants were harvested. The green weight of the freshly harvested plants, which have had their roots carefully separated from the agar and lightly blotted to free them of excessive moisture, was determined by a sensitive torsion balance. At the same time, the presence or absence of nodules in each case was recorded. An association between the inoculant and the host plant was deemed as " effective " if the growth of the host as judged by its green weight was more than 50% better than the uninoculated nitrate-free control.

(iii) *Determination of the " Most Probable Number " (MPN).*—Effective nodulation was recorded as positive, ineffective nodulation and absence of nodules were considered negative. The number of positives and negatives in respect of any three consecutive dilutions gives the " characteristic number " from which MPN is read off from the tables given by Calmette, Negre and Boquet (1948). The number of organisms in the original suspension is calculated from the formula $N = n \times d/V$, where N = number per ml. of the original suspension, n = most probable number based on the characteristic number and V = volume of suspension added to each tube and d = dilution represented by the first group of tubes used to determine the characteristic number.

(iv) *Determination of viable count.*—One ml. aliquots of serial dilutions 10^5 to 10^8 each of the inoculants in Ringer's solution were added to sterile petri plates to which yeast mannitol agar was added; incubated at 26° C. and plate counts of the rhizobia determined on the seventh day. The number of rhizobia per gram of the original sample on moisture-free basis is calculated. Only qualitative observations of the organisms other than rhizobia were made and recorded to represent the extent of contamination.

Results

The results of examination of the inoculants under study are presented in Table I. It is seen from the data:

(i) that the percentage of moisture of the samples is fairly constant and lies between 52–54%,

(ii) the viable count of rhizobia varied within wide limits;

(iii) the contaminants in a majority of cases exceeded the number of rhizobia;

(iv) the percentage effectiveness is more than 50% in all the samples studied.

DISCUSSION

The seedling agar tube method described here facilitates large-scale testing of inoculants for quality. As pointed out by Vincent (1954) it offers possibilities of judging the effectiveness of the association between the host and the rhizobia during the period of growth by observation of the nodulation pattern and the green colour of cotyledons and their relative speed of yellowing. The plants should be examined for this purpose when nitrate-free controls first show any yellowing of the cotyledons. At this time normally the nitrate controls as well as the inoculated plants will still have fully green cotyledons. It must be admitted, however, that the method is limited to the small-seeded legumes and to situations where maximum temperatures range between 70–80° F.

Besides the two types of controls provided in the experiments reported here, inclusion of strains of rhizobia of known performance as recommended by Vincent (1956) would provide additional check on the conditions of the experiment and permit better comparisons. Because of differences in response to different strains of rhizobia in species of the one cross-inoculation group (host-strain specificity) the two extreme species in each group are used, in the tests reported here, in duplicate tubes, *i.e.*, red and subterranean clover under clover group.

Four criteria were used to assess the quality of the inoculants: (i) the number of rhizobia per gram of the inoculant, (ii) degree of contamination by other organisms, (iii) extent of nodulation and (iv) effectiveness (Sankaram, 1958). It is quite obvious from the data presented here that neither the number of rhizobia per gram of the inoculant nor the degree of contamination present in these inoculants had any definite well marked effect on nodulation and effectiveness of the two clover species used in these tests.

SUMMARY

Samples of " peat-based " legume inoculants for clovers manufactured in Australia were examined in the laboratory for quality. The inoculants were examined to determine the number of rhizobia per gram of the inoculants, degree of contamination by other organisms, extent of nodulation and effectiveness, which formed the criteria to assess the quality. The study indicated that neither the number of rhizobia per gram of the inoculant nor

the degree of contamination had any marked effect on nodulation and effectiveness.

Acknowledgements

My sincere thanks are due to Mr. K. C. Marshall, Bacteriologist, N.S.W. Department of Agriculture, for helpful guidance and to Dr. C. J. Magee, Chief Biologist, for his keen interest in the work. I am grateful to the Commonwealth Government of Australia for the award of a fellowship under the Colombo Plan.

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CONTRIBUTION TO OUR KNOWLEDGE OF THE PHYSIOLOGICAL ANATOMY OF SOME INDIAN HYDROPHYTES

IX. The Stem of *Limnophila heterophylla* Benth.

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(Communicated by Dr. T. S. Sabnis, D.Sc., F.A.Sc.)

Introduction

Limnophila heterophylla Benth. is a large, heterophyllous aquatic herb, forming extensive and dense stands in swamps and on the shallow margins of ponds and lakes. It is capable of growing submerged in deep waters, as an emergent hydrophyte, or, as a wetland plant. The leaves of the completely submerged form are very finely dissected into several linear segments. Those of the emergent form show a distinct heterophylly (Fig. 1) which tends to become less pronounced in the wet-land plants. The bluish flowers are produced by the wet-land form and on the aerial parts of the shoots of the emergent one.

Material and Method

Collections were made from a number of localities round about Nagpur in different seasons of the year. The material was fixed in formalin acetic alcohol. A series of free-hand sections were taken at different heights of the stem of the emergent form. These were compared with the sections of the stems of the submerged and wet-land forms. The relative proportions of the cross-sectional areas of the different anatomical regions of the stems at various levels were determined by plotting their camera-lucida sketches against a graph-paper.

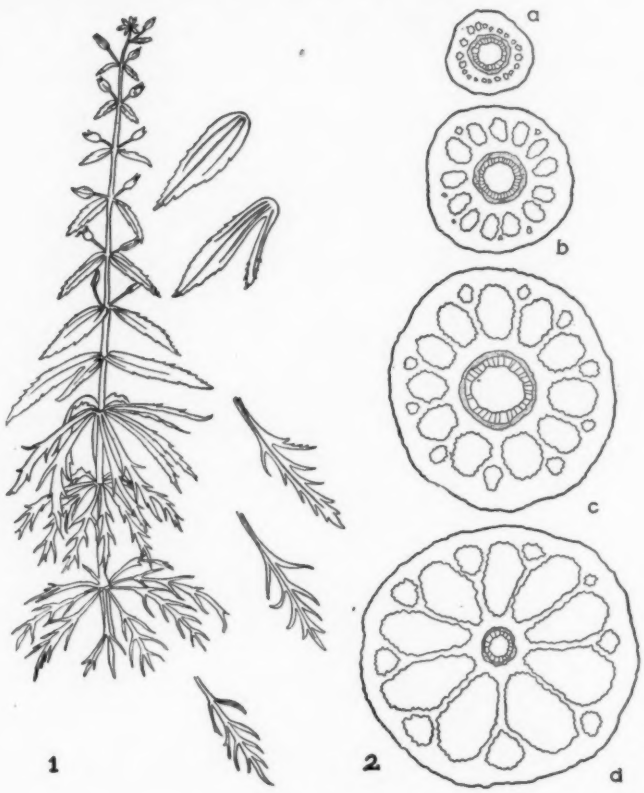
Description

The species has a creeping stem, rooting at the nodes and sending aerial shoots that protrude out of water if the water is very shallow. Plants growing in wet soil have erect stems.

Anatomical investigation of the stem reveals the following features of importance.

1. *Epidermis.*—Outer walls thickened; thin cuticle and few multicellular hairs on the aerial portions of the stem. Peltate multicellular glands frequent,

2. *Cortex.*—Outer cortical cells collenchymatous; collenchyma well developed in the aerial part (Fig. 3); cells elongated and with poorly thickened walls in the submerged portion (Fig. 4).

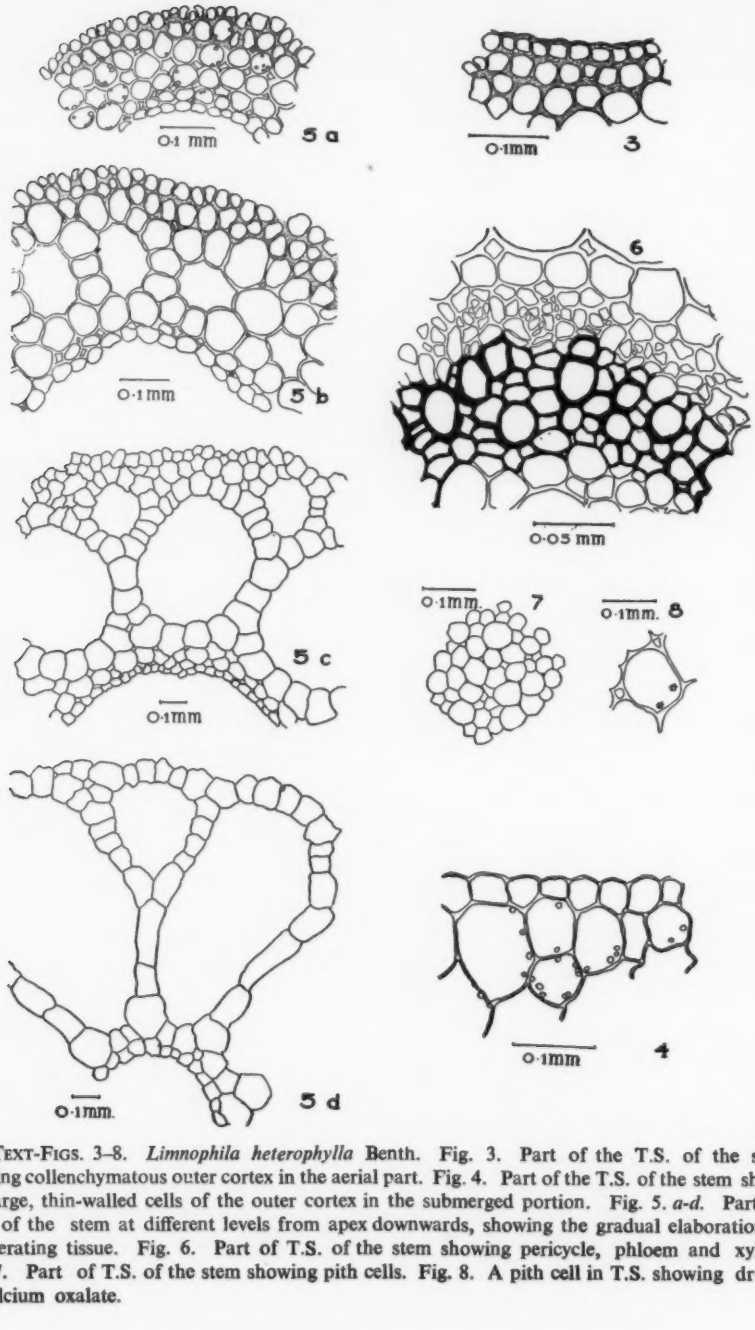


TEXT-FIGS. 1–2. *Limnophila heterophylla* Benth. Fig. 1. A part of the plant showing heterophylly. Fig. 2 *a-d*. A series of transverse sections of the stem showing relative proportions of the pith and the lacunar cortex.

Middle cortex chlorenchymatous and lacunar; some cells containing druses of calcium oxalate; air-spaces schizogenous, small and oval in the aerial part, very prominent and radially elongated in the submerged one (Fig. 5 *a-d*).

Endodermis distinct, cells containing plenty of starch.

3. *Pericycle.*—Thick-walled but not clearly distinguishable from the phloem elements below (Fig. 6); cells abound in starch.



TEXT-FIGS. 3–8. *Limnophila heterophylla* Benth. Fig. 3. Part of the T.S. of the stem showing collenchymatous outer cortex in the aerial part. Fig. 4. Part of the T.S. of the stem showing large, thin-walled cells of the outer cortex in the submerged portion. Fig. 5. *a-d.* Parts of T.S. of the stem at different levels from apex downwards, showing the gradual elaboration of the aerating tissue. Fig. 6. Part of T.S. of the stem showing pericycle, phloem and xylem. Fig. 7. Part of T.S. of the stem showing pith cells. Fig. 8. A pith cell in T.S. showing druses of calcium oxalate.

4. *Vascular system.*—The phloem and xylem form continuous concentric zones (Fig. 6). Xylem vessels arranged in radial rows. Stele very much reduced in extent in the submerged part.

5. *Pith.*—Thin-walled and parenchymatous (Fig. 7); cells showing druses of calcium oxalate (Fig. 8). The pith is extremely narrowed down in the submerged region of the stem, while the cortex becomes an increasingly prominent zone (Fig. 2 *a-d*).

Discussion

1. The general plan of structure of the stem does not differ essentially from that of the stems of other hydrophytes of this family described earlier, *viz.*, *Lindernia* All. (Mirashi, 1957) and *Bacopa monnieri* (Linn.) Pennell (Sayeedud-din, 1940). Mention has to be made, however, of the collenchymatous nature of the cortex, which is often not met with in the stems of Scrophulariaceæ. This provides a strong peripheral rim for the fragile lacunar cortex in the aerial part of the stem. The poor development of collenchyma and the reduction of the conducting tissue in the submerged stem indicate the weakening effect of water.

2. *The aerating system.*—This exists in the form of the primary lacunar cortex. In accordance with the greater degree of adaptation of this species to water, the aerating system is more elaborately developed than in the stems of the two wet-land plants mentioned above. As we pass from the aerial to the submerged part of the stem, there is a gradual widening of the cortex and a corresponding diminution in the extent of the pith. The ratio of the cross-sectional area of the pith to that of the cortex near the apex is 1 : 8, while in the submerged portions it becomes 1 : 160. This arrangement confers a two-fold advantage on the stem. In the first place, it brings about the concentration of mechanical elements nearer the centre in the submerged part, which is thus made inextensible. Secondly, there is a considerable increase in the space devoted to the aeration of the stem. Table I which gives a series of readings from the apex downwards, illustrates this fact in a striking manner.

3. The Scrophulariaceæ, to which the present species belongs, includes several hydrophytic genera exhibiting various degrees of adaptation to their habitat. The author (1957) has already described the physiological anatomy of the stems of certain wet-land species of *Lindernia* All. They have all been shown to possess a characteristic aerating tissue in the form of the lacunar cortex in their stems, indicating clearly their hydrophytic character. The present species, *Limnophila heterophylla* Benth., shows a much greater degree

TABLE I

$\frac{C}{E}$	Cross-sectional area occupied by the air-cavities / Area of the entire cross-section of the stem	$\frac{C}{E}$	Cross-sectional area occupied by the air-cavities / Area of the entire cross-section of the stem
1	0·0625	6	0·2564
2	0·0769	7	0·3226
3	0·1000	8	0·3717
4	0·1851	9	0·5263
5	0·2222		

of adaptation to the aquatic environment. It is even capable of growing submerged when in deep waters, although the flowers are only borne on shoots which may protrude out of water, when the water level has receded. The possession of heterophylly has helped towards a fuller adaptation to watery conditions. There seems to be no doubt that the dissected form of the submerged leaves is particularly well suited to life in water. Whatever be the origin of this feature, it seems certain that in the ecological evolution of Angiosperms some terrestrial species which were endowed with a strong tendency towards heterophylly could ultimately become aquatic. The more complete adaptation of this species to water is also borne out by its physiological anatomy. The morphological and physiological aids are supplemented by suitable anatomical arrangements for aeration.

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ALTERATIONS IN RAT SERUM PROTEINS IN RELATION TO DIETARY PROTEINS AND B VITAMINS

II. Studies during Protein Depletion and Repletion

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Received February 4, 1959

The literature on the influence of dietary protein quality and level on the rates of restoration of blood proteins in depleted animals is extensive (Madden and Whipple, 1940; Benditt *et al.*, 1941; Cannon *et al.*, 1943, 1944; Zeldis *et al.*, 1945; Wissler *et al.*, 1946; Chow *et al.*, 1948; Allison, 1955). Studies on the influence of dietary levels or deficiencies of B vitamins on these are, however, lacking. In the preceding paper of this series (Mulgaonkar and Sreenivasan, 1959) it was shown that B vitamins may influence the utilization of dietary amino acids (especially from low quality proteins) for the synthesis of serum proteins in the rat. The present work relates to observations on the effects of (i) certain B vitamins (thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid and vitamin B_{12}) on repletion of serum proteins and (ii) vitamin B_{12} and folic acid (PGA) on the degree of depletion of serum proteins and of hemoglobin, in the proteinfasted rat.

Experimental

Two series of experiments were carried out. Experiment 1 was aimed at ascertaining the influence of minimal and optimal levels of seven of the B vitamins on rates of repletion of serum proteins. Forty male Wistar rats, 115–120 g. in weight, were used. The animals originally subsisted on the laboratory stock diet consisting of (parts by weight): whole meal wheat flour 75, whole milk powder 2, casein 12, dried yeast 2, arachis oil 4, vitaminised sesame oil 1, sodium chloride 2 and calcium carbonate 2, the vitamin additions in the sesame oil providing vitamin A 500 I.U., α-tocopherol 5 mg., and vitamin K (Menadione, Merck) 1 mg.

Five animals were sacrificed to establish normal concentrations of serum protein fractions and the rest were reared on a protein-free diet consisting of (per cent.): maize starch 80, cellulose 3, sucrose 5, arachis oil 6, shark-liver oil 2, and salt mixture (U.S.P. No. XIV) 4. Vitamin additions carried by the

sucrose corresponded to (in mg. per kg. of diet): thiamine hydrochloride 6, riboflavin 10, nicotinic acid 30, calcium pantothenate 20, pyridoxine hydrochloride 6, PGA 5, vitamin B_{12} 0·25, biotin 1, *p*-aminobenzoic acid 100, choline chloride 500, inositol 500, vitamin K 10, and α-tocopherol 50.

At the end of two weeks, five animals were sacrificed to establish the serum protein profile in the depleted state. The rest were divided into two groups of fifteen each and re-fed the protein-free diet modified by the addition of vitamin-free casein (10 g. per 100 g.) at the expense of an equal amount of starch and with additions of the seven B vitamins at optimal or minimal levels. These were, respectively, as follows (in mg. per kg. of the diet): thiamine hydrochloride 3 and 0·75; riboflavin 4 and 1, nicotinic acid 20 and 5, calcium pantothenate 10 and 2·5, pyridoxine hydrochloride 3 and 0·75, PGA 1 and 0·03 and vitamin B_{12} 0·15 and nil. Vitamins other than these were added at the same levels as in the protein-free diet. Regeneration studies, with three rats from each group, were made at the end of 2, 4, 7, 11 and 14 days of *ad libitum* feeding.

In experiment 2, the effects, specifically, of a combined deficiency of vitamin B_{12} and PGA on depletion of serum proteins and hemoglobin during protein-fasting were studied.

Weanling male rats, 40 g. in weight, were divided into two groups of ten each, one of which received a basal vitamin B_{12}-deficient, Bengal gram (*Cicer arietinum*) diet. It has been observed in this and in other work from this laboratory (Lawate and Sreenivasan, unpublished) that a high-vegetable protein with Bengal gram is as effective as the corn-soybean ration (Register *et al.*, 1949) in producing vitamin B_{12} deficiency in rats. The basal diet consisted of (per cent.): Bengal gram 90, sesame oil 3, shark-liver oil 2, salt mixture (U.S.P. No. XIV) 4, and sucrose 1; vitamin additions were the same as in the protein-free diet of experiment 1 with omissions of vitamin B_{12} and PGA. The second group received this basal diet with supplements of vitamin B_{12} and PGA at levels of 200 μg. and 1 mg. respectively per kg. of the diet. Feeding was *ad libitum* for seven weeks. The average food intake of the deficient group over this period was about nine per cent. lower than that of the control group.

At the end of 7 weeks, five animals each from the deficient and control groups were sacrificed, while the rest were replaced on the original diets modified by withdrawal of Bengal gram and substitution by an equal amount of maize starch. Serum protein and hemoglobin changes were studied after one week of protein-fasting.

Preparation of serum

The animals were dissected under ether anesthesia. Blood was drawn from inferior vena cava using a dry syringe. A portion was immediately heparinized while the rest was allowed to clot at 37° for one hour in a sterile glass container. The separated serum was obtained by centrifugation at 2°.

Hemoglobin was estimated in 30 μl. portions of whole blood by acid hematin method.

Total serum protein was determined in 30 μl. portions of serum by the biuret method (Gornall *et al.*, 1949).

Serum protein fractionation was carried out by electrophoresis on Whatman No. 3 paper strips according to the procedure described earlier (Mulgaonkar and Sreenivasan, 1959). The relative concentrations of the protein fractions were estimated densitometrically using an 'EEL' Scanner (Evans Electroselenium Ltd., Harlow, Essex).

Results

Alterations in serum proteins due to protein depletion and during repletion with minimal and optimal intakes of B vitamins

The changes in body weight and in serum proteins due to protein-fasting of animals on stock diet are given in Table I. Typical electrophoretic patterns of serum proteins obtained before and after depletion are illustrated in Fig. 1. These data demonstrate marked reductions ($p < 0{\cdot}001$) in serum levels of albumin, α_1-globulin and β-globulin due to protein-fasting. While there is also a small, but insignificant ($p > 0{\cdot}2$), reduction in serum α_2-globulin level, γ-globulin level remains unaltered or is slightly increased numerically though not significantly ($p > 0{\cdot}2$); the albumin-globulin ratio is unaffected. During repletion, there is a faster restoration of total serum protein concentration with minimal than with optimal intake of B vitamins but growth is retarded (Fig. 2). In either group the total serum protein rises above the normal, pre-fasting, level but drops thereafter to levels below normal. This drop is more pronounced with minimal than with optimal intake of the B vitamins and also precedes the latter.

Figure 3 relates to the levels of serum protein fractions attained at periods during repletion with optimal and minimal intakes of the vitamins. In either group, during the first two days of regeneration, there is a small rise in the levels of α- and β-globulins with reductions in albumin and γ-globulin levels. With minimal intake of the vitamins the restoration of

TABLE I

Effects of protein-fasting on body weight and serum proteins

Forty male adult rats reared on laboratory stock diet were fed a protein-free diet for two weeks. Ten of these were sacrificed, five before and five after protein depletion, for serum protein fractionation studies. Others were used for regeneration studies. Change in body weight due to protein-fasting are for all thirty-five rats. All figures are mean values ± standard deviations.

Group	Body weight (g.)	Serum proteins					
		Total protein	Albumin	α_1-globulin (g./100 ml.)	α_2-globulin	β-globulin	γ-globulin
Normal	119·0 ± 3·0	6·80 ±0·11	2·82 ±0·02	1·31 ±0·09	0·67 ±0·05	0·89 ±0·01	1·09 ±0·02
Depleted	88·0 ± 6·4	5·40 ±0·34	2·12 ±0·16	0·86 ±0·04	0·56 ±0·16	0·69 ±0·03	1·16 ±0·11

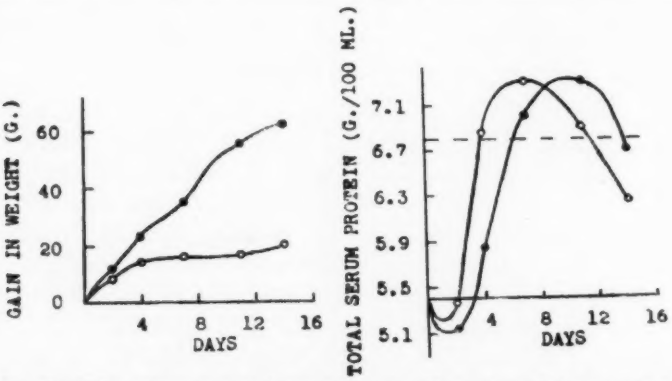

FIG. 2. Repletion of serum proteins and growth. Normal rats fed a protein-free diet for two weeks (see Table I) were re-fed a 10 per cent. casein diet at optimal (●) or minimal (o) levels of thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid and vitamin B_{12}. The broken line indicates the serum protein level of animals before protein depletion; the origin denotes the level after depletion. Each of the plots on the curves represents the average value of at least 3 determinations.

albumin and α_1-globulin is incomplete; on the other hand, there is a marked rise in β- and γ-globulins to abnormal levels. However, at the end of two weeks, the β-globulin level returns to normal, while there is some drop in the γ-globulin level also. The restoration of α_2-globulin is rapid and complete.

With optimal intake of the vitamins the serum protein fractions attain normal levels in the course of two weeks. The albumin concentration, in good parallelism with the total protein level, decreases after 11th day of repletion to below normal level following an initial increase. α_1-Globulin is almost completely restored after 14 days. α_2-Globulin increases slightly above normal in 4 days and maintains this level during subsequent period. β-Globulin, which initially shows an increment similar to, but less marked than, that observed in the low B vitamins-fed group, returns to normal at the conclusion of the regeneration period. The γ-globulin level fluctuates within the normal range. Figure 4 demonstrates the differences in the electro-phoretic profile due to variations in intake of the B vitamins, as at the end of the repletion period.

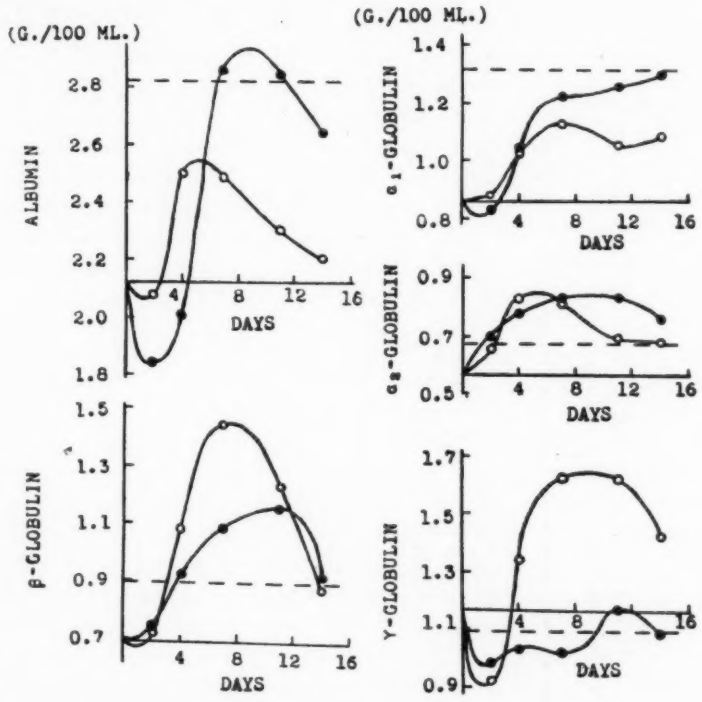

FIG. 3. Changes in electrophoretically separated serum protein fractions at various stages in the course of regeneration in protein depleted rats fed a 10 per cent. casein diet with optimal (●) or minimal (o) levels of seven B vitamins. Details as in Table I and Fig. 2. The broken line indicates the level of the protein fraction before depletion; the origin denotes the level after depletion. Plots represent in each case the average of three determinations.

TABLE II

Effects of vitamin B_{12} and PGA on the depletion of blood proteins

Weanling rats were reared on a vitamin B_{12}-deficient basal Bengal gram diet with or without supplements of vitamin B_{12} and PGA for seven weeks. The animals were then protein-depleted for one week. Blood hemoglobin and serum protein levels were determined before and after depletion. Figures represent mean values ± standard deviation. There were five rats in each series.

Group	Body weight (g.)	Hemoglobin (g./100 ml.)	Serum proteins (g./100 ml.)					
			Total protein	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin
Before Depletion								
Basal	213·0 ± 24·0	11·80 ± 0·62	6·27 ±0·28	2·26 ±0·06	1·18 ±0·06	0·72 ±0·11	1·10 ±0·07	0·99 ±0·11
Basal + Vitamin B_{12} + PGA	280·0 ± 16·6	11·81 ± 0·47	7·20 ±0·24	2·67 ±0·10	1·39 ±0·10	0·68 ±0·09	1·33 ±0·06	1·11 ±0·12
After Depletion								
Basal	198·8 ± 19·2	10·62 ± 0·34	5·00 ±0·36	1·67 ±0·07	0·81 ±0·06	0·48 ±0·06	0·85 ±0·04	1·18 ±0·10
Basal + Vitamin B_{12} + PGA	266·5 ± 11·8	11·46 ± 0·38	6·45 ±0·08	2·37 ±0·08	1·15 ±0·06	0·57 ±0·10	1·17 ±0·05	1·17 ±0·08

Influence of vitamin B_{12} and PGA on blood proteins during protein-fasting

In Table II are shown the changes in body weight, hemoglobin and serum proteins due to protein-fasting of rats on the basal Bengal gram diet and the effects on these of vitamin B_{12} and PGA. It is seen that supplements of the vitamins have positive effects on growth and on serum protein concentration but not on the hemoglobin level. The higher serum protein concentration of the vitamin supplemented group is reflected in elevated levels of albumin ($p < 0{\cdot}001$), α_1-globulin ($p < 0{\cdot}005$) and β-globulin ($p < 0{\cdot}001$).

Protein fasting brings about marked reductions in hemoglobin ($p < 0{\cdot}01$) and in total serum protein ($p < 0{\cdot}001$) concentrations in the deficient animals. The effects are less severe in the group receiving vitamin B_{12} and PGA with no significant change in hemoglobin ($p > 0{\cdot}2$), and a smaller, though significant, reduction in total serum protein ($p < 0{\cdot}001$), even though animals in the two groups have lost equally in weight. There are marked reductions ($p < 0{\cdot}005$) in serum levels of albumin, α_1-globulin and β-globulin in either group. Additionally, there is a decrease in α_2-globulin level ($p < 0{\cdot}005$) and a small but significant rise in γ-globulin level ($p < 0{\cdot}02$) in the deficient animals. These changes bring about marked alterations in the relative distribution of protein fractions in the deficient animals with decrease in the relative concentrations of albumin, α-globulins and β-globulin and an increase in γ-globulin proportion. On the other hand, the serum protein pattern of rats receiving vitamin B_{12} and PGA show a striking constancy of distribution.

Discussion

The present observations on the effects of protein-fasting on rat serum proteins are in accord with those of Allison (1955) and also with our previous findings in rats on 18 and 10 per cent. casein diets (Mulgaonkar and Sreenivasan, 1959) that a reduction in dietary protein level chiefly affects serum levels of albumin, α_1-globulin and α_2-globulin. Moreover, it is seen that the reductions are most striking in the albumin and α_1-globulin levels. This supports our earlier postulation that the dependence of albumin and α_1-globulin fractions on dietary amino acids is greater than that of other serum protein fractions. On the other hand, failure to observe any appreciable change in γ-globulin concentration with variations in dietary protein quality and level (Mulgaonkar and Sreenivasan, 1959), the latter amounting to protein-fasting in the present experiments, would suggest that its synthesis is least susceptible to qualitative or quantitative variations in dietary amino acids. Although similar conclusions have been reached by other workers with respect to antibodies and γ-globulin production in rats on low protein diets (Metcoff

et al., 1948; Wertman and Sarandria, 1952; Wertman *et al.*, 1952; Allison, 1955), and in under-fed humans (Bieler *et al.*, 1947; Balch, 1950; Keys *et al.*, 1950), it is possible that the degree of depletion is an important factor here, severe protein depletion having been found to affect both γ-globulin and antibody synthesis in dogs (Cannon *et al.*, 1943, 1944; Zeldis *et al.*, 1945; Chow *et al.*, 1948).

Since the animals received adequate amounts of B vitamins in the protein-free diet it is understandable why, during regeneration, growth and serum protein repletion progressed for a considerable period even though the intake of the B vitamins was minimized. Thus, the rise in the levels of albumin and α-globulins regressed only after 4 days of protein re-feeding.

The faster replenishment of total serum protein level with minimal than with optimal intake of B vitamins is associated with retarded growth and may indicate a preferential channelling of amino acids into serum protein synthesis in this condition. It is to be noted, however, that this faster repletion occurs through abnormal rise in β- and γ-globulin levels. A similar behaviour of these globulins has been recorded earlier in protein depleted dogs re-fed an enzymatic hydrolyzate of casein (Chow *et al.*, 1948). That this is a specific property of casein was evident as a lactalbumin hydrolyzate failed to produce a similar effect whereas an alcoholic extract of casein had a globulin-regenerating action when added to the lactalbumin hydrolyzate (Albanese *et al.*, 1950). Other investigators (Holman *et al.*, 1934; Madden and Whipple, 1940; Seeley, 1945) have also noted a similar behaviour of the plasma globulin fraction upon feeding casein to protein-depleted dogs.

The observed parallelism between growth rate and serum albumin level brings out an interesting relationship between protein nutriture and serum albumin concentration. The final level attained by the different protein fractions in the control group is probably a function of dietary protein quality and level. It is of interest to note that a similar trend in albumin and β-globulin levels is observed in rehabilitating starving subjects (Keys *et al.*, 1950).

In our studies mentioned above, growth retardation in rats on an 18 per cent. casein diet with minimal supplements of B vitamins is attended by a decrease in serum levels of α_1- and γ-globulins. The partial restoration of α_1-globulin and the ultimate drop in the level of γ-globulin encountered presently, during repletion with minimal B vitamins, are in accord with these findings. It is possible that the incomplete restoration of albumin, observed additionally, is due, at least in part, to the low level of protein in the diet.

The improved growth and blood picture resulting from supplementation of the basal Bengal gram diet with vitamin B_{12} and PGA may be attributed

to the known sparing effect of the vitamins on methionine, which is the limiting amino acid in Bengal gram protein (Vijayaraghavan and Srinivasan, 1953). The failure to obtain any increase in hemoglobin level upon vitamin supplementation, as against a definite increase in serum protein concentration, is similar to the findings of Hsu *et al.* (1953) that in the young chick fed a 65% soybean protein diet dietary vitamin B_{12} increased the plasma protein level but not the hemoglobin level.

Vitamin B_{12} and PGA apparently moderate the effects of protein-fasting on hemoglobin and serum proteins. A similar effect of vitamin B_{12} on blood amino acids has been described by Charkey and co-workers (1954) who found that the rise in the free amino acids of the blood in fasting chicks was moderated by vitamin B_{12} at levels in the diet above that required for maximal growth.

Summary

Protein-fasting for two weeks of rats on the laboratory stock diet results in a decrease in serum level of albumin, and of α_1-, α_2- and β-globulins. The albumin-globulin ratio remains unaltered.

Repletion on a 10 per cent. casein diet with minimal as compared to optimal intake of the B vitamins—thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid and vitamin B_{12}—causes a faster restoration of total serum protein but growth is retarded. With minimal intake of the vitamins there is an incomplete restoration of albumin and α_1-globulin; an abnormal initial increase in β- and γ-globulins is followed, upon prolonged regeneration up to two weeks, by a drop in all fractions and in total serum protein. With optimal intake of B vitamins, the fractions attain normal levels at or before two weeks of regeneration. An initial abnormal increase in serum β-globulin resembles that observed at minimal intake of the vitamins. Following prolonged regeneration there is a drop in albumin and in total serum protein to subnormal levels, which effects are attributable to low level of protein in the diet.

Rats on a basal, vitamin B_{12} deficient, Bengal gram diet show reductions in blood hemoglobin, serum albumin and in serum α_1-, α_2-, and β-globulins upon protein-fasting for one week. The effects are moderated with supplements of vitamin B_{12} and folic acid to the basal diet. Incidental observations also point to growth-promoting and serum protein-generating effects of the vitamins in the growing rat.

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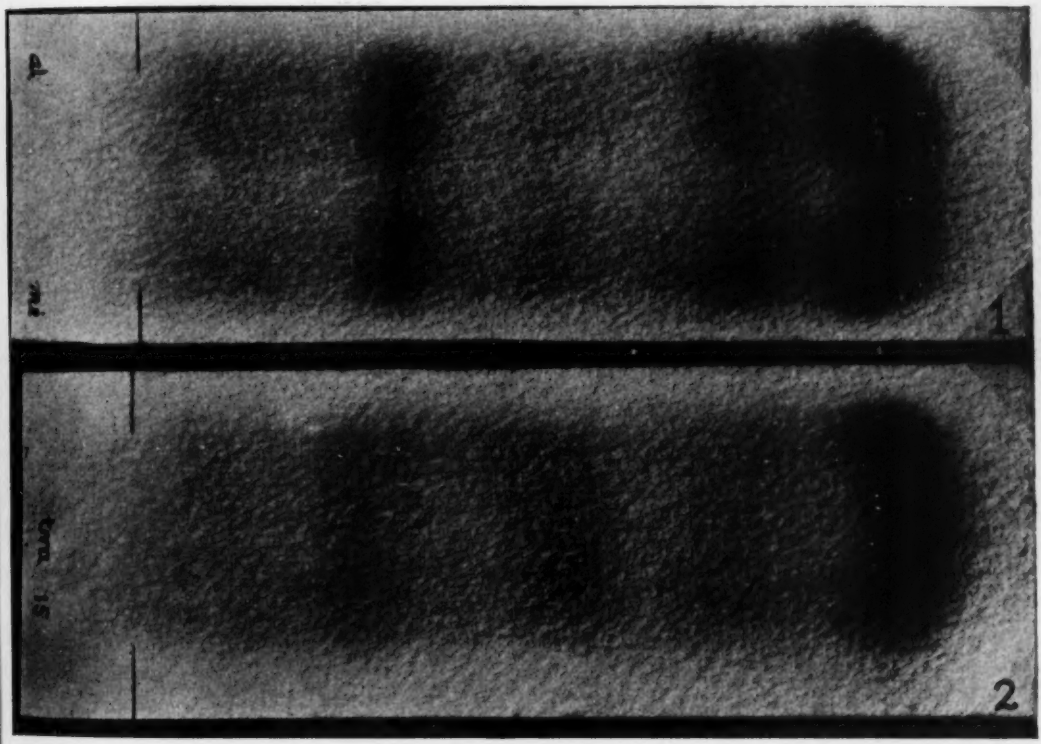

FIG. 1. Alterations in rat serum proteins due to protein fasting. Pattern 1 is a typical electrophoretic profile with ormal animals. Alterations due to protein fasting for two weeks, are seen in Pattern 2. Details as in Table I. The bands ading from starting line are, in order, γ-globulin, β-globulin, α_2-globulin, α_1-globulin and albumin.

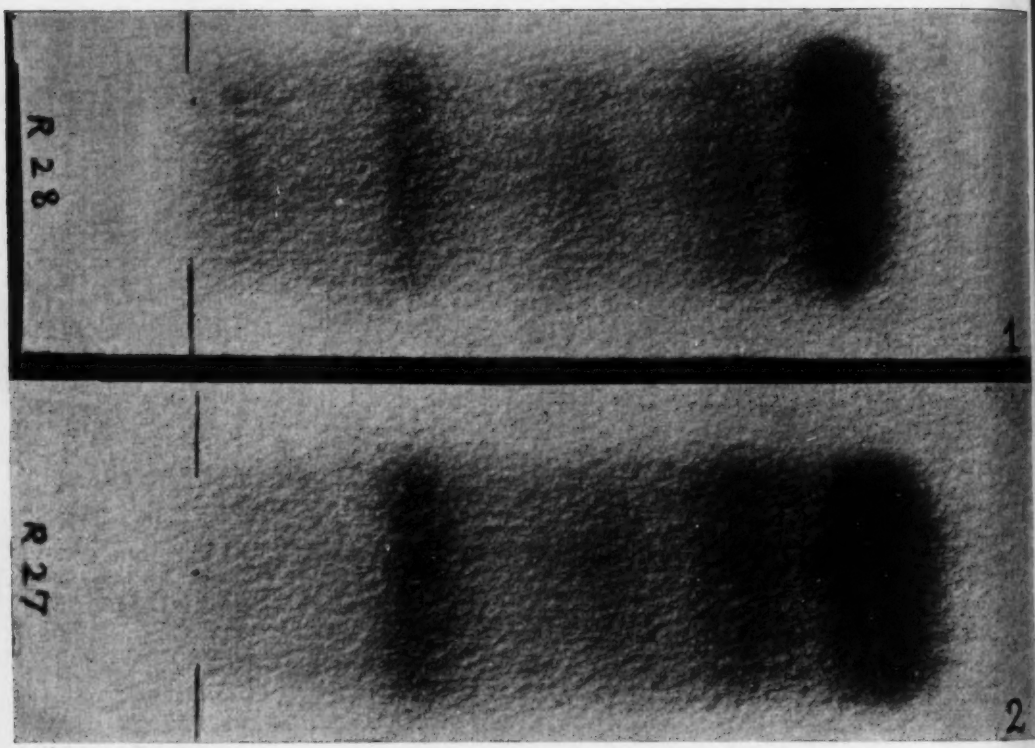

FIG. 4. Restoration of serum proteins in protein-depleted rats upon feeding a 10 per cent. casein diet, at minim
'attern 1) or optimal (Pattern 2) levels of seven B vitamins for 2 weeks. For details of the experiment refer Fig. 2. Th
sposition of the protein bands in the patterns follows the order given in Fig. 1.

STUDIES ON THE NUTRITION OF RICE PLANT (*ORYZA SATIVA* L.)

Part VI. Utilization of Ammonium and Nitrate Nitrogen by Rice Plant Under Water-logged Soil State

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Tanaka *et al.* (1959 *c*) reported that under moderate supply of nitrogen, ammonium and nitrate nitrogen were equally good sources of nitrogen for grain yield of rice plant, grown under solution culture. Better crop growth and grain yield was, however, reported with ammonium nitrogen given at the vegetative growth and nitrate nitrogen, later at elongation and reproductive stage. Dastur and Malkani (1933) also stated that rice plant preferred ammonium nitrogen at early stage and nitrate nitrogen at later stage of growth. In soil culture, under water-logged soil conditions, however, nitrate has been found to be an inferior source of nitrogen for rice crop (Sethi *et al.*, 1952; Grist, 1953 and Abichandani and Patnaik, 1958 *a*).

Comparison between fertilizer efficiency of ammonium and nitrate nitrogen, for increasing rice yields under field condition have been done mostly with basal application of fertilizer, given either full dose at planting or a few days after planting. Mixture of ammonium and nitrate fertilizers has also been tried by Dastur and Pirzada (1934), who considered the mixture superior to either of the two forms used alone. Influence of ammonium and nitrate fertilizers, applied singly at vegetative and reproductive stages respectively or one form following the other at later growth period has, however, not been tried. In view, therefore, of the results reported by Dastur and Malkani (1933) and Tanaka *et al.* (1959 *c*), from solution culture investigations that ammonium nitrogen is better source of nitrogen for rice crop at early stages of growth and nitrate nitrogen at later stages, soil culture experiments were undertaken to study the utilization of nitrogen by rice plant from ammonium and nitrate fertilizers in relation to their time of application.

Material and Methods

A medium duration variety T 141 of 145 days maturation period was transplanted on July 25 in earthenware pots, each containing 10 kg. of rice soil. Three hills, with one seedling each, were planted per pot. Soil used

was clay loam having pH of 6·0. It contained 0·08 per cent. total nitrogen, 0·085 per cent. organic carbon.

The two sources of nitrogen were given as ammonium sulphate and sodium nitrate respectively, at the rate of 0·70 gm. nitrogen per pot. This level of nitrogen was considered, from previous experiences, as adequate for growth under pot conditions. In addition, each pot received 0·50 gm. each of P_2O_5 and K_2O as superphosphate and potassium sulphate respectively.

Nitrogen fertilizers were given both as single and split application. In single application, full nitrogen level of 0·70 gm. in one or the other form of nitrogen, was given either basal at planting or was top-dressed at later stages of growth. Split application of nitrogen was done as $\frac{3}{4}$ nitrogen, given basal at planting as ammonium fertilizer and $\frac{1}{4}$ top-dressed at later stages of plant growth either as ammonium or as nitrate fertilizer. Top-dressing was tried at two stages of plant growth, either on September 4, about 15–20 days before ear-initiation or on October 7, at boot stage, about 10–12 days before flowering. Single application treatments are designed under experimental results (Tables I, II, III and IV) as 0·70–0·00–0·00 or 0·00–0·70–0·00 or 0·00–0·00–0·70 and split application as 0·47–0·23–0·00 or 0·47–0·00–0·23, figures denoting quantities of fertilizer nitrogen basal applied or top-dressed, later on September 4 and October 7 respectively.

Observations on plant growth were recorded at weekly intervals. Three pots in each treatment were kept for yield and additional pots, two each were also kept for sampling on September 4 and October 7, before each top-dressing. Crop was harvested on November 22. Harvest plants and samples obtained at earlier stages of growth were all studied for dry matter weight and nitrogen content. Nitrogen in the plant samples was determinded by micro-Kjeldahl method, after air-drying and powdering the plant material through a Willey grinding mill.

EXPERIMENTAL RESULTS

(*a*) *Plant height and tiller number.*—Data on plant height and tiller number per plant for respective treatments at harvest are shown in Table I. Single application of ammonium fertilizer, given at different stages of plant growth, is seen to be far superior to that of nitrate fertilizer. Weekly growth data for single application of ammonium fertilizer, given at different stages of growth is represented in Fig. 1 (*a* and *b*). Application of ammonium fertilizer is seen to have remarkable effect both on plant height and tiller number per plant. Full dose of ammonium fertilizer, given at pre-ear-initiation stage on September 4, is seen to increase both plant height and tiller number

TABLE I

Influence of nitrogen application on height and tiller number per plant at harvest

Treatment	Mean plant height cm.	Mean tillers per plant	Mean ear-bearing tillers per plant
1. Control ..	97	4·2	4·2
2. Single application			
A. Ammonium fertilizer:			
0·47–0·00–0·00	105	6·8	6·8
0·70–0·00–0·00 ..	107	9·5	9·5
0·00–0·70–0·00 ..	115	8·7	8·2
0·00–0·00–0·70 ..	104	7·7	6·6
B. Nitrate fertilizer:			
0·70–0·00–0·00 ..	99	4·3	4·3
0·00–0·70–0·00 ..	104	5·2	5·2
0·00–0·00–0·70 ..	104	4·2	4·2
3. Split application over basal ammonium fertilizer			
A. Ammonium fertilizer—top-dressed:			
0·47–0·23–0·00 ..	114	7·8	7·5
0·47–0·00–0·23 ..	108	7·3	6·8
B. Nitrate fertilizer—top-dressed:			
0·47–0·23–0·00 ..	108	7·3	7·3
0·47–0·00–0·23 ..	102	6·6	6·6

more than that obtained with full dose applied basal at planting, but all the late-developed tillers, however, do not put up ear heads, and ear-bearing tillers at harvest, therefore, with application at pre-ear-initiation stage is slightly less than that with basal application. Ammonium fertilizer, given at the boot stage, also increases plant height and tiller number, but the increase is not so marked as that with earlier applications.

Single application of nitrate fertilizer, either basal at planting or applied at later stages of growth, has practically no effect on plant growth. Application of full dose of nitrate fertilizer at pre-ear-initiation stage only, when the plant is in active vegetative growth, however, gives a slight increase in tiller number per plant over control.

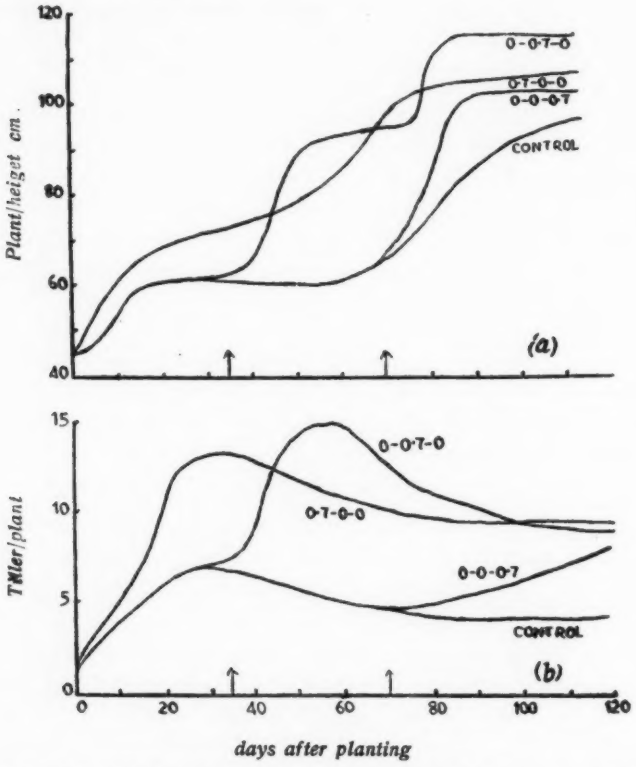

FIG. 1. Influence of single application of ammonium nitrogen on growth of rice plant (↑ indicates time of fertilizer application).

With split application of nitrogen, $\frac{2}{3}$ given basal as ammonium fertilizer and $\frac{1}{3}$ later top-dressed as ammonium or nitrate fertilizer, a slight gain in plant height is observed with ammonium fertilizer. There is, however, no difference in ear-bearing tillers per plant, whether top-dressing is done with ammonium or nitrate fertilizers. Weekly growth data for split application of nitrogen for ammonium fertilizer is shown in Fig. 2 (*a* and *b*). It is seen that split application of fertilizer gives slightly taller plants but number of ear-bearing tillers per plant, obtained with split application, is less than that

given by full dose ammonium fertilizer, applied basal at planting or at pre-ear-initiation stage. Of the two top-dressings done at pre-ear-initiation and boot stages respectively, the former is seen to be superior in increasing plant height and tiller number than the latter.

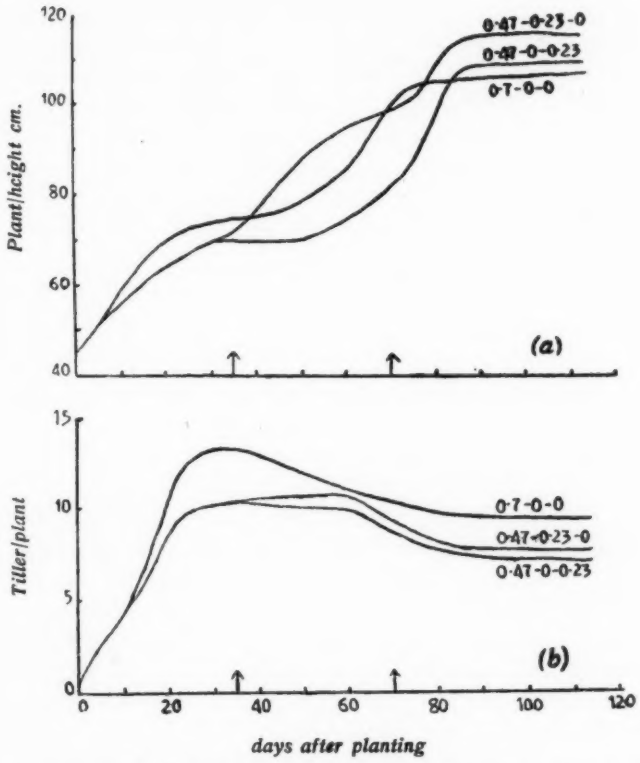

FIG. 2. Influence of split application of ammonium nitrogen on growth of rice plant (↑ indicates time of fertilizer application).

(*b*) *Dry matter weight and nitrogen content of crop at harvest.*—Table II shows ear-bearing tillers per pot, dry matter weight and nitrogen content of ear and straw, for different treatments at harvest. Mean panicle weight for each treatment is also shown. These results also show that nitrate fertilizer is an inferior source of nitrogen to ammonium fertilizer, for increasing rice yields. Nitrate fertilizer, applied full dose at planting or at boot stage, has practically no effect on crop yield. Application at pre-ear-initiation stage, however, inc reases crop yield, but only slightly. Ammonium fertilizer, on

TABLE II

Influence of nitrogen application on dry matter weight and nitrogen content of crop at harvest

Treatment	Mean panicles per pot	Dry matter weight (gm./pot)		Mean panicle weight gm.	N %	
		Ear	Straw		Ear	Straw
1. Control ..	12·5	14	16	1·12	1·05	0·42
2. Single application ..						
A. Ammonium fertilizer:						
0·47–0·00–0·00	20·5	32	46	1·56	0·89	0·33
0·70–0·00–0·00	28·5	40	60	1·40	0·90	0·36
0·00–0·70–0·00	24·5	42	51	1·71	0·97	0·42
0·00–0·00–0·70	20·0	23	29	1·15	1·65	0·87
B. Nitrate fertilizer:						
0·70–0·00–0·00	13·0	16	20	1·23	1·00	0·39
0·00–0·70–0·00	15·5	23	29	1·48	0·96	0·42
0·00–0·00–0·70	12·5	17	18	1·36	1·14	0·51
3. Split application over basal ammonium fertilizer						
A. Ammonium fertilizer—top-dressed:						
0·47–0·23–0·00	22·5	43	58	1·91	0·90	0·36
0·47–0·00–0·23	20·5	41	49	2·00	1·00	0·39
B. Nitrate fertilizer—top-dressed:						
0·47–0·23–0·00	22·0	40	44	1·82	0·88	0·39
0·47–0·00–0·23	20·0	38	41	1·90	0·93	0·35

the other hand, increases crop yield remarkably, the increase being more with higher level of nitrogen used. Application of ammonium fertilizer, full dose (0·70 gm. N) at pre-ear-initiation stage, gives slightly more ear yield but comparatively low straw yield than basal application at planting.

Ammonium fertilizer, given at boot stage, is not found to be very effective in increasing crop yield.

With split application of nitrogen, higher total crop yield is obtained with ammonium fertilizer, $\frac{2}{3}$ given basal at planting and $\frac{1}{3}$ later top-dressed at pre-ear-initiation stage. Top-dressing of ammonium fertilizer at boot stage gives slightly less ear yield but very much reduced straw yield than top-dressing at pre-ear-initiation. Nitrate fertilizer top-dressed is also seen to give slightly lower ear yield but far less straw yield than ammonium fertilizer top-dressed.

Yield increase with nitrogen application is seen to be generally accompanied with increase in mean panicle weight and with split application of fertilizer, top-dressing further increases mean panicale weight (Table II). It is seen that although less ear-bearing tillers per pot are obtained with split application than with basal application at planting or at pre-ear-initiation stage, ear yield is same or slightly more because of higher mean panicle weight, obtained in these treatments.

The cause of increase in panicle weight due to top-dressing was analysed by study of spikelet number and ratio of unfilled to filled grains per panicle and weight of 1,000 grains in each of the treatments with ammonium nitrogen. For this study, ten panicles, five heavier and five lighter than the mean panicle weight, were chosen at random from each treatment. Results are given in Table III. It is seen that with full nitrogen dose (0·70 gm. N), applied at pre-ear-initiation stage, as compared to full dose given at planting, total spikelet numbers per panicle increase remarkably in the heavier panicles, which were formed on tillers put up at earlier growth period of the plant. Late developed lighter panicles are not benefited. Ratio of number of chaff to filled grains per panicle is also lowered in the heavier panicles, by late application of the fertilizer. The late-developed lighter panicles, on the contrary, are seen to put up more chaffiness due to late nitrogen application.

Top-dressing of fertilizer over basal application is, on the other hand, seen to benefit both the earlier developed heavier panicles and late-developed lighter panicles. Total spikelet numbers per panicle increases by top-dressing and also the ratio of number of chaff to filled grain decreases for both types of panicles. Weight of 1,000 grain is also seen to increase when fertilizer application is split up and top-dressing is done at pre-ear-initiation or at boot stage.

Nitrogen content (Table II) of no nitrogen plants is slightly higher than those that received basal or pre-ear-initiation fertilizer application. Nitrogen content of the plant is, however, seen to increase remarkably with delay in

nitrogen application and highest nitrogen content in the ear and straw is obtained with full dose of ammonium fertilizer, applied at boot stage.

TABLE III

Influence of nitrogen application on panicle character

(Character of light and heavy panicles from ammonium fertilizer treatments)

Treatment	Panicle size	Total spikelets per panicle	Chaff number / Filled grain No. × 100	1,000 grain weight gm.
1. Control ..	Light	151	28	19·2
	Heavy	541	30	19·0
2. Single application				
0·47–0·00–0·00 ..	Light	250	28	19·5
	Heavy	671	23	19·0
0·70–0·00–0·00 ..	Light	291	39	19·0
	Heavy	654	22	19·2
0·00–0·70–0·00 ..	Light	262	58	18·7
	Heavy	836	15	19·5
0·00–0·00–0·70 ..	Light	192	88	15·7
	Heavy	604	7	18·5
3. Split application over basal ammonium fertilizer:				
0·47–0·23–0·00 ..	Light	325	27	19·8
	Heavy	769	11	19·7
0·47–0·00–0·23 ..	Light	377	12	20·6
	Heavy	701	6	20·0

(*c*) *Nitrogen uptake.*—Data on nitrogen absorbed (mg./pot) by crop at different stages of growth and utilization of fertilizer nitrogen by crop during its life-cycle are given in Table IV. Nitrogen absorbed at various stages of plant growth is evaluated from dry matter weight and nitrogen content of crop at that stage. Utilization of fertilizer nitrogen for each treatment is worked out by subtracting the amount of total nitrogen absorbed by crop without nitrogen application from that with respective nitrogen treatments.

TABLE IV

Total nitrogen absorbed by crop at different stages of growth and fertilizer nitrogen absorbed by crop at harvest

Treatment	Nitrogen absorbed by crop (mg./pot)			Fertilizer nitrogen absorbed		Top-dressed nitrogen absorbed	
	Up-to 4/9 before first top-dressing	Up-to 7/10 before second top-dressing	Up-to 23/11 at harvest	mg.	%	mg.	%
1. Control ..	125	187	231	..	..	..	..
2. Single application							
A. Ammonium fertilizer:							
0·47–0·00–0·00	403	413	471	240	51	..	..
0·70–0·00–0·00	584	576	619	388	55	..	..
0·00–0·70–0·00	125	595	653	422	60	..	..
0·00–0·00–0·70	125	187	652	421	60	..	..
B. Nitrate fertilizer:							
0·70–0·00–0·00	145	210	262	31	4	..	..
0·00–0·70–0·00	125	313	368	137	20	..	..
0·00–0·00–0·70	125	187	305	74	11	..	..
3. Split application over basal ammonium fertilizer							
A. Ammonium fertilizer—top-dressed:							
0·47–0·23–0·00	403	598	644	413	59	173	75
0·47–0·00–0·23	403	413	644	412	59	172	75
B. Nitrate fertilizer—top-dressed:							
0·47–0·23–0·00	403	485	555	320	46	84	37
0·47–0·00–0·23	403	413	528	297	42	57	25

Total nitrogen uptake by crop with ammonium fertilizer is seen to be nearly twice as much as with nitrate fertilizer, at each stage of growth. With ammonium fertilizer, total nitrogen uptake by the crop is about the same, irrespective of time of application, although crop yield with nitrogen application at boot stage is very much less. The nitrogen applied at this stage of growth is seen to accumulate in the plant tissue (Table II) and increases the nitrogen content of the crop. With split application of nitrogen also, total nitrogen uptake is more when ammonium fertilizer is top-dressed than when nitrate fertilizer is top-dressed.

Results of utilization of fertilizer nitrogen by crop show that about 55–60 per cent. of the applied fertilizer nitrogen is absorbed with ammonium fertilizer, while about 4–20 per cent. only is absorbed with nitrate fertilizer. With split application also, 59 per cent. of fertilizer nitrogen is utilized with ammonium fertilizer, given at both stages of growth and only about 42–46 per cent. is utilized when ammonium fertilizer is given as basal dressing and nitrate fertilizer is top-dressed. Utilization of top-dressed fertilizer is seen to be about 75 per cent. with ammonium fertilizer and about 25–37 per cent. with nitrate fertilizer.

DISCUSSION

Results presented show the comparative merit of ammonium and nitrate fertilizers, for increasing rice yields under submerged soil state. Contrary to earlier findings from solution culture studies, reported by Tanaka *et al.* (1959 *c*), nitrate nitrogen alone applied to rice crop at any stage of growth is found to be an inferior source of nitrogen for growth of rice under submerged soil condition. With split application of nitrogen, $\frac{2}{3}$ given basal as ammonium sulphate and $\frac{1}{3}$ top-dressed later, either as ammonium or nitrate fertilizer, also, comparatively less total crop yield is obtained with nitrate fertilizer top-dressed. There is, however, very little difference between the two sources of top dressed nitrogen as regards ear yield of the crop is concerned, but nitrate fertilizer top-dressed gives very much lower straw yield than ammonium fertilizer top-dressed. Results regarding the low fertility value of nitrate fertilizer for increasing rice yields under submerged soil state have been earlier reported by Kelley (1911), Bartholomew (1929), Sethi *et al.* (1952) and from experiments conducted at the Central Rice Research Institute (Ghose *et al.*, 1956). The reason for low fertility value of nitrate fertilizer is because, the utilization of fertilizer nitrogen by rice crop is seen to be far greater with ammonium fertilizer than with nitrate fertilizer. With former, about 50–60 per cent. of the applied fertilizer nitrogen is utilized and with latter, only 4–20 per cent. is utilized with different

times of application. Utilization of top-dressed nitrogen is also found to be higher with ammonium fertilizer than with nitrate fertilizer. The low utilization of nitrate fertilizer by rice crop under submerged soil state may be attributed to non-availability of nitrogen in the soil for crop utilization from this fertilizer. In the submerged soil state, because of anaerobic conditions prevailing in the lower layer of soil, nitrogen losses occur from applied fertilizers due to denitrification (Pearsall, 1950; Shioiri and Tanada, 1954 and Mitsui, 1955). These losses have been found to be comparatively more with nitrate fertilizer than with ammonium fertilizer. Jansen and Metzger (1928), De and Digar (1954) and Abichandani and Patnaik (1958 *b*) have all reported more losses of nitrogen from nitrate fertilizers than from ammonium fertilizers, applied under submerged soil state. Disappearance of nitrate nitrogen from water-logged soils within 7–15 days of water-logging has also been shown by De and Sarkar (1936), Bhuiyan (1949), Takai *et al.* (1956) and Yamane (1957). Low fertility value of nitrate fertilizers, occurring under anaerobic condition of rice cultivation may therefore be attributed to denitrification and subsequent disappearance of nitrate nitrogen from soil.

Nitrate nitrogen applied at later stages of growth, however, appears better utilized than that applied basal at planting. It has been suggested by Mitsui (1955) that the denitrification process, which is active in the flooded soil in the initial stages of submergence, weakens as conditions settle down under prolonged flooding. It follows therefore that nitrate fertilizer applied at later growth stages should be more available for crop utilization than applied earlier at planting. Besides this, the plant at later stages of growth is in active vegetative stage with more root surface and is, therefore, in a position to absorb nitrogen from nitrate fertilizer before the same is denitrified. Nitrogen utilization from top-dressed nitrate fertilizer (0·23 gm. N) is about 25–37 per cent., while that from single application (0·70 gm. N), applied at similar stages of growth, is only about 11–20 per cent. This shows that the plant which has received earlier basal application of fertilizer, being in better stage of growth, is able to utilize more nitrogen from applied nitrate fertilizer than the plant highly deficient in nitrogen. Therefore, nitrate fertilizer, if it is to be given to rice plant, should be given only as a top-dressing over basal application of ammonium fertilizer.

Figure 3 illustrates the influence of nitrogen source, nitrogen level and time of application on different plant characters. Since nitrate fertilizer was found inferior for increasing yield, results for ammonium fertilizer are only illustrated. Best time of nitrogen application for optimum ear yield

is seen to be split application of fertilizer, given $\frac{2}{3}$ basal at planting and $\frac{1}{3}$ later top-dressed just before ear-initiation. Tanaka *et al.* (1959 *b*) have pointed out that rice crop utilizes nitrogen vigorously at two stages of growth, the vegetative stage and the ear development stage. It has also been suggested

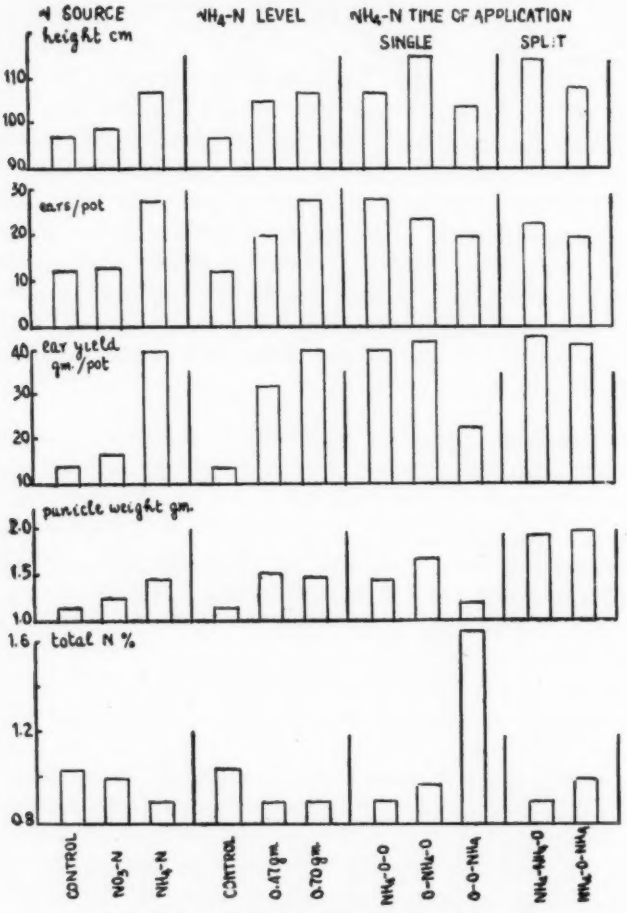

FIG. 3. Influence of nitrogen application on plant characters.

by Tanaka *et al.* (1959 *b*) that full dose of fertilizer, applied to soil at early stages of growth, may not always be available for later growth, because of leaching and denitrification conditions prevalent in submerged rice soils and therefore need for second application later may be felt. Split application of nitrogenous fertilizer would therefore appear as a rational means of com-

bating nitrogen losses in submerged soils and thus increase the nitrogen availability and rice yields.

Results also show that top-dressing at pre-ear-initiation stage or boot stage increases mean panicle weight. This is in accordance with observations made earlier by Tanaka *et al.* (1959 *a*), that increased nitrogen uptake during ear development increases panicle weight. It is seen that although split application of fertilizer results in the production of less ear-bearing tillers per pot than full dose applied basal at planting, it gives slightly higher ear yield because of increase in panicle weight. The reason for increase in panicle weightdue to top-dressing is seen to be the increase in spikelet number and lower ratio of unfilled to filled grain. One thousand grain weight is also increased by top-dressing of the fertilizer over basal application.

Summary

Comparative fertilizer efficiency of ammonium and nitrate fertilizers, applied at different stages of growth, to rice crop, grown under submerged soil state, has been investigated. Single and split application of fertilizers given basal at planting, at pre-ear-initiation stage and at boot stage respectively have been tried. Following conclusions have been arrived at:—

(*a*) Ammonium fertilizer, applied to rice crop at any stage of growth is superior to nitrate fertilizer for increasing rice yields. Percentage utilization of nitrogen from ammonium fertilizer varies between 55 and 60 per cent. with different times of application while with nitrate fertilizer, it varies between 4 and 20 per cent.

(*b*) Nitrate fertilizer, top-dressed over a basal dressing of ammonium fertilizer, gives only slightly less ear yield than ammonium fertilizer top-dressed, but gives far less straw yield than the latter. Percentage utilization of nitrogen by crop from top-dressed fertilizer is 75 with ammonium fertilizer and 25–37 with nitrate fertilizer.

(*c*) Single application of ammonium fertilizer, either basal at planting or at pre-ear-initiation stage gives about the same ear yield but comparatively higher straw yield is obtained with basal application at planting. Fertilizer application at boot stage only is not as effective for increasing rice yields as earlier applications.

(*d*) Split application of ammonium fertilizer, $\frac{2}{3}$ given basal at planting and $\frac{1}{3}$ later top-dressed at pre-ear-initiation stage, is found slightly advantageous over full dose, given basal at planting or at pre-ear-initiation stage. The slight increase in ear weight, obtained with split application, is seen to be due to increase in mean panicle weight, obtained because of top-dressing

at later stage of growth. The increase in mean panicle weight is found to be due to increase in the number of set grains per panicle and 1,000 grain weight.

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STUDIES IN PECTIC ENZYMES OF PARASITIC FUNGI

II. Factors Affecting the Production of Protopectinase Enzyme by *Rhizopus stolonifer* (Ehrenh. ex. Fr.) Lind.

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Introduction

Rhizopus stolonifer (Ehrenh. Ex. Fr.) Lind. has been found to cause serious rot disease in plums in the market of Naini Tal and to infect many other fruits (Bhargava and Gupta, 1957). Harter and Weimer (1921 and 1923 *b*) made some observations on the protopectinase (definition given by Kertesz, 1951) enzyme produced by this organism while they were working out details of the enzyme of *Rhizopus tritici*. In the present work the effect of factors, like media, incubation temperature and incubation period on the extracellular production of protopectinase enzyme by *R. stolonifer* has been reported.

Materials and Methods

The strain of *Rhizopus stolonifer* used in this work was locally isolated from rotten plums.

For the preparation of protopectinase enzyme the fungus was grown on natural and synthetic liquid media.

Natural liquid media were decoctions of various fruits, seeds or other parts of the plants prepared by boiling 200 gm. of it for 45 minutes in distilled water, filtered through muslin and the filtrate made up to 1 litre.

The enzyme solution was also prepared by infecting surface sterilized ripe fruits using the method given by Bhargava and Gupta (1957) and extracting the sap from the rotten tissues after the incubation period.

The following synthetic liquid media were prepared:

(*a*) *Dextrose pectin medium.*—25 gm. pectin, 20 gm. ammonium nitrate, 2 gm. dextrose, 0·02 gm. magnesium sulphate ($MgSO_4.7\,H_2O$) and 10 gm. of potassium dihydrogen phosphate (KH_2PO_4) in 1 litre of distilled water.

(*b*) *Pectin asparagin medium.*—Five gm. glucose, 10 gm. pectin, 4 gm. asparagin, 10 gm. calcium carbonate powder, 0·5 gm. magnesium sulphate and 1 gm. potassium dihyrogen phosphate in 1 litre of distilled water.

(*c*) *Modified Czapek's medium.*—3 gm. sodium nitrate, 0·5 gm. potassium chloride, 15 gm. sucrose, 15 gm. pectin, 0·5 gm. magnesium sulphate, 1 gm. potassium dihydrogen phosphate and 0·01 gm. ferrous sulphate in 1 litre of distilled water.

Only 15 ml. of a culture medium was taken in each flat bottle of 12 fluid oz. capacity and sterilized by autoclaving for 20 minutes at 15 lb. pressure. Each bottle was inoculated by transferring a few spores along with some mycelium from 15 days old stock culture. The spores were evenly distributed in the culture medium by shaking the bottles. The latter were then stacked on their flat sides in incubators at various temperatures. After the incubation period the fungal mat was removed from the surface of the culture, the liquid was centrifuged and used as such for protopectinase enzyme.

The activity of protopectinase enzyme in a solution was tested by the potato disc method of Brown (1915) and has been expressed here as the time taken to macerate 3 potato discs of 11 mm. diameter and 0·5 mm. thickness when kept in 3 ml. solution of the enzyme preparation.

pH values were determined by using Hellige pH Comparator.

EXPERIMENTAL

(i) *Enzyme production on infected fruits.*—Seven kinds of ripe fruits were inoculated and incubated at 25° C. for 5 and 9 days. The activity of protopectinase enzyme in the extracted sap from the rotten tissues has been shown in Table I.

All the seven kinds of fruits which were inoculated were found to be susceptible though apple was not so rapidly infected as other fruits. Brinjals, peaches, pears and apricots were most readily infected and the activity of the protopectinase enzyme also was found to be best in the rotten tissues of these fruits.

(ii) *Enzyme production on natural media.*—Decoctions of different fruits, potato tubers, carrots and lucerne (seeds of *Medicago sativa*) were prepared, inoculated and incubated at 25° C. The activity of the protopectinase enzyme was tested after 5 days incubation period. It was observed (Table II) that protopectinase enzyme of sufficient activity could not be obtained on any of the natural medium. Harter and Weimer (1923 *a*) working with *Rhizopus tritici* reported that the prune decoction was inferior to the decoctions of potato and carrot for the production of the enzyme.

In the present case decoction of potato and carrot were not found to form a better medium for the enzyme production than prune decoction though the

TABLE I

Enzyme production on different fruits

Fruit	5 days incubation		9 days incubation	
	% of rot	M.T.[1]	% of rot	M.T.
Apple ..	20	12 hrs.[2]	58	4–6 hrs.
Apricot ..	98	110 mts.[3]	100	..†
Brinjal ..	100	55 mts.	The fruit dried	..
Kakoo* ..	80	12 hrs.	100	..†
Peach ..	100	35–40 mts.	100	60 mts.
Pear ..	100	120 mts.	100	190 mts.
Plum ..	50	> 12 hrs.	100	6–8 hrs.

* Fruit of *Diospyrous kaki* (Persimmon).
† Bacterial infection.
[1] M.T. = Maceration time for standard potato discs.
[2] hrs. = hours.
[3] mts. = minutes.

TABLE II

Enzyme production on different natural media

Medium	Initial pH	Final pH	M.T.
Marrow* ..	7·4	7·6	>24 hrs.
Papaya ..	7·1	7·6	>24 hrs.
Peach ..	4·3	4·0	12–13 hrs.
Plum ..	<3·8	<3·8	12–13 hrs.
Tomato ..	4·2	7·6	>24 hrs.
Potato ..	7·6	8·2	>24 hrs.
Carrot ..	7·2	7·6	>24 hrs.
Lucerne ..	7·1	8·2	12–18 hrs.

* Fruit of *Cucurbita pepo* var. *blighia sapida*.

latter was also not very satisfactory. Lucerne medium, which was reported to be most suitable for *Penicillium notatum* (Lulla and Johar, 1953), did not prove to be so with the present fungus. As the living fruits form a good medium for the secretion of enzyme (Table I), there is possibility that natural media if prepared by different methods might give better results (Weurman, 1953; Cole, 1956 and Gupta, 1958).

(iii) *Enzyme production on synthetic media.*—Only dextrose pectin medium (A), pectin asparagin medium (B) and modified Czapek's medium (C) were used for enzyme preparation. All these media have been reported to be suitable for different organisms for pectic enzyme secretion by different workers (Lulla and Johar, 1953; Singh and Wood, 1956 and Harter and Weimer, 1923 *a*). Cultures were incubated at 25° C. for 3 and 5 days. Results are shown in Table III.

TABLE III

Enzyme secretion on synthetic media with KH_2PO_4

Medium	Initial pH	3 days incubation		5 days incubation	
		Final pH	M.T.	Final pH	M.T.
A ..	2·8	<2·8	>24 hrs.	<2·8	>24 hrs.
B ..	7·0	8·2	>24 hrs.	8·3	>24 hrs.
B without $CaCO_3$	3·3	3·7	80 mts.	8·5	20–22 hrs.
C ..	2·8	2·8	>24 hrs.	3·0	>24 hrs.

In another set of experiments media A, B and C of Table III were slightly modified by replacing the potassium dihydrogen phosphate with the equal amount of tribasic potassium phosphate to form media A_1, B_1 and C_1 respectively. The results are shown in Table IV.

It was noted that only pectin asparagin medium without the addition of calcium carbonate powder was best suited to *R. stolonifer* for protopectinase enzyme secretion. In the case of *Bacterium aroideæ* (Wood, 1955) and *Fusarium moniliforme* (Singh and Wood, 1956) the importance of $CaCO_3$ in the medium was emphasised, while it was reported by Gupta (1956) that addition of Ca ion in the medium is harmful for the enzyme secretion by *Pythium debaryanum*. In the present case the addition of $CaCO_3$ in the medium reduced the quality of the medium. In general the addition of tri-

basic phosphate in place of dihydrogen phosphate improved the medium for protopectinase enzyme secretion.

TABLE IV

Enzyme secretion on synthetic media with K_3PO_4

Medium	Initial pH	3 days incubation		5 days incubation	
		Final pH	M.T.	Final pH	M.T.
A_1 ..	6·8	4·3	5–6 hrs.	4·0	6–7 hrs.
B_1 ..	7·6	..	5–6 hrs.	..	>24 hrs.
B_1 without $CaCO_3$	3·9	4·2	60 mts.	8·4	8–9 hrs.
C_1 ..	3·3	3·2	>24 hrs.	3·2	>24 hrs.

(iv) *Effect of incubation temperature on enzyme production.*—In a set of experiments the cultures were grown on pectin asparagin medium (without $CaCO_3$) and incubated at 4 different temperatures for 3 days. It was noted that incubation at 25° C. is best suited for extracellular protopectinase enzyme production by *R. stolonifer* (Table V), though Harter and Weimer (1921) showed that 20° C. incubation temperature was best for the enzyme production by the same fungus when grown on sweet potato decoction. However, the present results are in general agreement with those of the authors mentioned that higher temperatures decrease both the activity of the enzyme and the growth of the fungus.

TABLE V

Effect of incubation temperature on enzyme production

Incubation temperature ° C.	Initial pH	Final pH	M.T.
20	3·3	3·3	110–120 mts.
25	3·3	3·7	80 mts.
28·5	3·3	3·3	>24 hrs.
30	3·3	3·3	..*

* No growth of the fungus.

(v) *Effect of incubation period on enzyme production.*—Using pectin asparagin medium (without $CaCO_3$) and incubating the cultures at 25° C. from 1–10 days, the activity of the enzyme production and changes in pH of the medium were followed (Fig. 1). It was noted that the activity of the

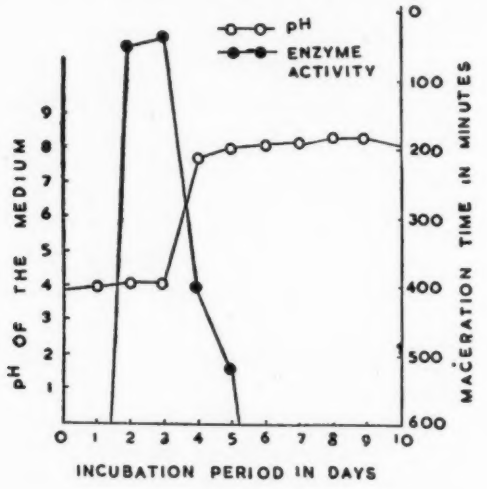
pH
ENZYME ACTIVITY
pH OF THE MEDIUM
MACERATION TIME IN MINUTES
INCUBATION PERIOD IN DAYS
0 1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9
0 100 200 300 400 500 600

FIG. 1

enzyme produced by the fungus suddenly increased after 24 hours growth of the fungus reaching its maximum in 3 days after which it rapidly fell. At the same time it was observed that there was only little rise in the pH of the medium for 3 days but suddenly increased afterwards. It might be that in the present case the pH of the medium influences the protopectinase activity or its production. Futher work is under progress on this problem.

SUMMARY AND CONCLUSIONS

Rhizopus stolonifer (Ehrenh ex . Fr.) Lind. secretes active protopectinase enzyme while attacking the plant tissues. Decoctions of several fruits and vegetables were not found to form suitable medium for the enzyme secretion. Pectin-asparagin medium (without $CaCO_3$) was found to be satisfactory for the enzyme secretion when cultures were incubated at 25° C. for 2–3 days.

ACKNOWLEDGEMENTS

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STUDIES ON THE EFFECT OF BETA RADIATIONS ON INSECTS

I. The Effect of Beta Radiations (Irradiation of Eggs and 1st Instar Larvæ) on the Life-history of *Corcyra cephalonica* Stainton

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Introduction

Very little published information is available on the effects of beta radiations on insects. Butler (1936) has reviewed the major effects of nuclear radiations on developing animal structures, like inhibition or retardation of growth and differentiation as well as the production of atypical organs. Similar effects of beta radiations from radioactive phosphorus have been reported by Martin, Dent and Joseph (1948) in a parasitic wasp, *Habrobracon juglandis* Ashmead and by Blumel (1950) in two species of *Drosophila*. It is evident that the nuclear radiations cause considerable damage to the biological tissues, as these highly energetic radiations penetrate the body tissues and dissipate energy to the latter. But the probability of tissue being damaged may be little with low levels of radiation exposures since the size of the nucleus is smaller as compared to that of a tissue cell and consequently the probability of nuclear radiations striking the nucleus will be low with low levels of radiation intensities (Bleuler and Goldsmith, 1952). If the nuclear radiations do not hit the nucleus of a tissue cell, there will not be any destruction of the latter. Keeping this in view the present work was undertaken to study the effects of different intensities of beta radiations on the development of *Corcyra cephalonica* Stainton which is a useful laboratory host for several beneficial hymenopterous parasites like *Bracon gelechiæ* Ashmead, *B. hebetor* Say, *B. brevicornis* Wesmael and *Apanteles angaleti* Muesebeck, etc.

Material and Methods

The eggs and larvæ of *C. cephalonica* were taken from the mass culture maintained in the Parasitology Laboratory of the Division of Entomology. The beta radiations from phosphorus (P^{32}) source were used in this investigation. Radioactive phosphorus (P^{32}) decays into a stable isotope of sulphur (S^{32}) with the emission of beta particles having the maximum energy of

1·71 Mev. and an average energy of 0·695 Mev. The physical half life of P^{32} is 14·3 days. The radioactive isotope of phosphorus was supplied by the Radiochemical Centre, Amersham, England, in the form of phosphoric acid ($H_3P^{32}O_4$).

Requisite volumes of radioactive phosphoric acid containing 0·5, 1, 2 and 3 millicuries of P^{32} were pipetted on to one inch diameter aluminium planchets and were then dried under an infra-red lamp. The planchets were then covered with a cellophane paper to avoid accidental radioactive contamination. The absorption of the radiations by the cellophane covering (11·14 mg./cm.^{2}) was negligible. The cellophane covered side of the planchets was kept parallel and facing the bottom of the glass jars (4 inches in diameter and 6 inches in height) where the freshly laid eggs and freshly hatched larvæ were kept in petri dishes for irradiation. A distance of 12 cm. was maintained between the test material and the P^{32} source on the planchet in all the experiments. The radiation exposure dosages were calculated after Bleuler and Goldsmith (1952), who have shown that one millicurie source of P^{32} gives at a distance of one metre a radiation dose of 16 mrep per hour. On the basis of this, P^{32} sources of 0·5, 1, 2 and 3 millicuries activities at a distance of 12 cm. thus give dosages of 555, 1111, 2222 and 3333 mrep/hr. respectively.

Batches of 250 eggs laid by the females within four hours were spread uniformly on the bottom of petri dishes (3 inches diameter), the latter being placed in the irradiation jars kept at 29 ± 4° C. and 70 ± 4% R.H. They were exposed for 24 hours to beta radiations from sources of radioactive phosphorus giving dosages of 555, 1111, 2222 and 3333 mrep/hr. Then the eggs were placed singly for hatching in specimen tubes (3 inches × 1 inch) closed with perforated corks. The freshly hatched larvæ were fed on crushed Jowar mixed with 10% yeast. They later on pupated and the adults of the first generation emerged out, which were separated into pairs of male and female which were kept separately in specimen tubes for oviposition. The eggs laid daily were kept separately for hatching. The newly hatched larvæ were reared in glass jars (6 inches × 4 inches) containing crushed jowar mixed with 10% yeast. Similarly the life-history of the insect in the second generation was also studied.

In another set of experiments 100 freshly hatched larvæ of *C. cephalonica* were similarly irradiated for 24 hours with beta radiations giving the same dosages and the life-history up to two successive generations was studied. Unirradiated eggs and larvæ were kept as controls and their subsequent generations were simultaneously studied. All the life-history studies were carried out at 29 ± 4° C. and 70 ± 4% R.H.

EXPERIMENTAL FINDINGS

The observations from the foregoing experiments on the irradiation of eggs are incorporated in Table I and those of the larvæ in Table II. It is evident from the data in Table I that there is no embryonic mortality due to beta radiations in the parent generation in any of the treatments, as the per cent. viability of the eggs even in the treatment with a high dose of 3333 mrep/hr. is almost equal to that in the control. There is also no detrimental effect of these intensities of beta radiations on the development of the caterpillars hatching out of the treated eggs as the per cent. successful completion of development even in the highest dose is almost equal to that of the control.

TABLE I

	Treatment dose (mrep/hr.)				Contro
	555	1111	2222	3333	
Parent generation					
No. of eggs exposed ..	250	250	250	250	250
Per cent. larvæ hatched ..	40·4	64·0	39·2	26·8	29·2
Per cent. larvæ pupated ..	52·0	85·6	71·4	76·1	65·7
Per cent. adults emerged	71·1	86·8	82·9	76·4	81·3
Per cent. successful completion of development	14·8	47·6	23·5	15·6	15·6
First generation					
Total No. of eggs laid ..	2957	1628	2396	838	1665
No. of females ..	13	8	10	4	7
Fecundity per female ..	227	203	239	209	223
Per cent. viability ..	11·36	51·3	11·95	26·85	17·52
Per cent. adults emerged	58·93	30·45	95·39	76·43	62·36
Per cent. successful completion of development	6·7	16·22	9·52	20·52	10·15
Second generation					
Total No. of eggs laid ..	2299	1487	2957	3173	2339
No. of females ..	11	10	11	11	10
Fecundity per female ..	209	149	268	286	234
Per cent. viability ..	14·03	26·36	30·10	13·23	13·28
Per cent. adults emerged	45·29	43·36	25·28	33·10	34·0
Per cent. successful completion of development	6·35	11·43	7·61	4·38	4·70

On the other hand, the 1111 and 2222 mrep/hr. dosages are more effective than the other two dosages as the per cent. successful completion of development in these two dosages is more than in the others. However, 1111 mrep/hr. dose is more effective than the 2222 mrep/hr. dose since the per cent. successful completion of development is distinctly higher in the former.

Dent and Amy (1950) have observed that when the eggs of *H. juglandis* are irradiated for 48 hours with beta radiations from radioactive phosphorus sources having activities between 1200 and 2100 microcuries, there is either no hatching or there is mortality in the larval stage. However, most of the individuals become imagoes when the eggs are exposed to low levels of radiation intensities (68–200 microcuries). This is probably due to: (*a*) The exposure period is longer (48 hours) and the distance between the radiation source and the eggs in their experiments is less than 2 mm. with the result that the dosages to which the eggs have been subjected are considerably higher than those used in the present investigation and, (*b*) the test insects were different in both the cases.

In the first generation the per cent. viability of eggs is again higher with 1111 mrep/hr. dose, whereas the per cent. adult emergence is more with 2222 mrep/hr. dose; but the per cent. successful completion of development increases in the case of 3333 mrep/hr. which is not markedly different from that of 1111 mrep/hr. dose. Further in the second generation the per cent. successful completion of development is higher with 1111 mrep/hr. dose, but there is no marked difference amongst different dosages and the control.

In the case of freshly hatched larvæ which are irradiated with the same intensities of beta radiation, the per cent. successful completion of development in parent generation is higher in all the treatments as compared to the control. Moreover, amongst the treatments it is distinctly higher with 1111 mrep/hr. dose (Table II). In the subsequent development of the two generations there is no apparent effect of these intensities of beta radiations as there is not much difference in the per cent. successful completion of development of the insect in the treatments and the control.

Contrary to the findings of Martin, Dent and Joseph (1948) and Blumel (1950) the present findings have indicated that exposure of the eggs and the first instar larvæ to the beta radiations does not appear to be detrimental, and the effect is dependent on the intensities of beta radiations. A dose of 1111 mrep/hr. is beneficial so far as the development of *C. cephalonica* is concerned. It, therefore, appears that the effect of these intensities of beta radiations from radioactive phosphorus (1111 mrep/hr.) on the eggs and

TABLE II

	Treatment dose (mrep/hr.)				Contr
	555	1111	2222	3333	
Parent generation					
No. of larvæ exposed ..	100	100	100	100	100
Per cent. adults emerged	69	85	65	62	48
Per cent. successful completion of development	69	85	65	62	48
First generation					
Total No. of eggs laid ..	2808	2713	3490	2518	2405
No. of females ..	11	11	11	10	9
Fecundity per female ..	255	247	317	252	267
Per cent. viability ..	40·9	20·1	25·04	32·9	27·6
Per cent. adults emerged	24·1	45·6	26·2	34·0	39·1
Per cent. successful completion of development	9·9	9·1	6·5	11·1	10·6
Second generation					
Total No. of eggs ..	2363	1147	1642	1971	2089
No. of females ..	11	9	11	9	11
Fecundity per female ..	215	123	149	219	190
Per cent. viability ..	41·9	27·3	38·3	30·9	19·8
Per cent. adults emerged	34·0	45·8	27·1	32·1	53·4
Per cent. successful completion of development	14·2	12·5	10·4	9·9	10·6

first instar larvæ of *C. cephalonica* is stimulating in nature, incidental rather than cumulative in character.

SUMMARY

The irradiation of eggs and first instar larvæ of *Corcyra cephalonica* Stainton with beta radiations from P^{32} has shown that even a dose as high as 3333 mrep/hr. does not produce any detrimental effect on the subsequent development of the parent as well as the two successive generations. However, the per cent. viability and per cent. successful completion of development in the parent generation are comparatively high with 1111 mrep/hr. dose than with 555, 2222 and 3333 mrep/hr. dosages and the control. Thus it may be concluded that the beta radiations of 1111 mrep/hr. intensity seem to be of stimulating nature for the eggs and larvæ of this insect.

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ON THE RENAL UNIT IN SOME COMMON TELEOSTS*

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Introduction

The numerous teleostean fishes inhabiting our coastal waters and estuaries, although normally restricted within definite ranges in salinity, show wide differences in their ability to endure changes in salt content of the environment. A few species among these are relatively more euryhaline and, on account of their remarkable powers of adaptation, are highly useful for culture in saline lagoons and coastal fish farms where seasonal fluctuation in salinity is a usual phenomenon. In view of the important role played by the excretory organs in the maintenance of water and salt balance in the body and the possible changes in their structure, the significance of a careful study of the renal units in these fishes, among other things, need hardly be overemphasised. Thus, this work was started as a basic means of approach to the wider problem on adaptation in some of the fishes as applicable to salt-water fish culture. However, in this preliminary report the scope has been circumscribed to cover only a brief description of the histologic characteristics of the renal units of a few selected species which might serve as a helpful background for experimental studies on adaptational physiology.

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Considering the vast amount of literature from other countries dealing with the structure of the kidney in fishes it may be said that such basic information with regard to several of our teleosts is lacking. Extensive discussions on the subject are available in a series of earlier papers beginning with the classical anatomical study by Audigé (1910) and others too numerous to be included here. Periodic reviews by workers like Smith (1932) and Marshall (1934) have covered various aspects, particularly dealing with the functional morphology of the kidney. The recent book by Smith (1953) entitled *From Fish to Philosopher* is a valuable contribution as it contains a bibliography with summaries of subsequent work on the subject besides a fascinating account of the whole question of evolution of the kidney in relation to environment. In view of these comprehensive reviews and a brief summing up by the same author later in 1954, a detailed historical resume at present seems superfluous.

The author is grateful to Dr. N. K. Panikkar for suggesting this line of investigation and to Dr. S. Jones for his constructive criticism.

Material and Methods

The material includes a few of the more common species of teleosts occurring along the coast and estuaries, obtained from the fishing nets either alive or immediately after death. The kidney from adult fish was dissected out in the field itself and a uniform procedure of fixing the material soon after dissection was adopted. Some care was exercised in the selection of the fixative as comparative measurements were to be made and corrosive-formol was used in all cases as this was found to give the best result compared to many other fixatives. Sections were taken from the posterior half of the kidney at a thickness of 5–7 micra and usually stained with Heidenhain's iron hæmatoxylin counterstained with eosin. Mallory's aniline blue stain was also helpful in differentiating the parenchymatous lymphoid tissue and blood capillaries clearly. In giving the diameter of the glomeruli in sections the procedure by Marshall and Smith (1930) was followed and hence the same limitations are applicable. No other strictly quantitative data are given in comparisons except in a general manner, as for example, the obvious differences in the relative amount of lymphoid tissue in some kidneys. Similarly, a rough idea of the distribution of the glomeruli in kidneys is given after taking their average number in 20 widely separated random sections in the series. Graphic method of reconstruction of the renal tubules was not very successful. Fresh pieces of the kidney was macerated in a 1 : 1 solution of hydrochloric acid and distilled water for 7–14 hours and a fairly

satisfactory preparation of the major portion of the tubules was obtained in some cases which helped in the study of the general structure of the tubules.

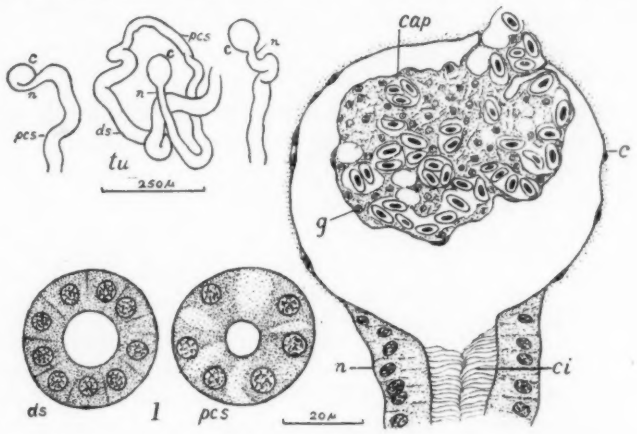
OBSERVATIONS

The fish kidney is made up of numerous renal units as in other vertebrate kidney and whose anatomical structure is basically the same in all the species. Each unit is composed of a glomerulus enclosed in a capsule and connected to a coiled uriniferous tubule. The major part of the tubule may be sometimes divisible into a proximal convoluted segment nearer to the glomerulus and a distal segment opening into the collecting duct. But in many of the marine species this distinction may not be present. The space among these highly coiled tubules is filled by parenchymatous lymphoid tissue with rich ramifications of blood capillaries. The more important anatomical features and histology of the renal units in a few species of fish are given below:—

1. *Megalops cyprinoides* (Fig. 1).—The glomerulus has an average diameter of 64 micra together with the capsule which is composed of extremely flat or squamous epithelial cells and a glomerular tuft consisting of finely divided capillaries. A wide space is often observed within the capsule as the capillary tuft does not completely fill the capsule. At the junction of the glomerulus with the uriniferous tubule a neck region can be distinguished and which varies in length in the different tubules as shown in three typical cases (*tu*) obtained in maceration preparations. The neck is characterised by a narrow ciliated lumen of about 8 micra and the cells show an average height of 10 micra with uniformly staining cytoplasm and nucleus having rich, deeply staining chromatin granules. The rest of the uriniferous tubule is divisible into two regions of nearly equal lengths. In the initial regions the lumen is relatively narrow with a diameter of only 6 micra and the cells have large round nuclei situated nearer to the base of the cells. Distally the lumen becomes wider with an average diameter of 12 micra where the cells are more cuboidal with an average height of 7 micra and having almost centrally placed nuclei. Cilia are absent throughout the length of the uriniferous tubule except in the neck and the histology of the tubule reveals that the distal region is distinct from the proximal segment and that the former corresponds to the distal convoluted segment in the renal tubules of the freshwater species.

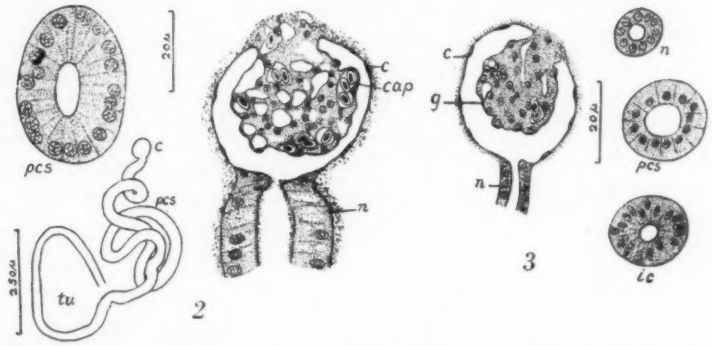
2. *Chanos chanos* (Fig. 2).—The glomerulus measures on an average 47 micra in diameter possessing a highly branching capillary tuft almost filling the capsule. A slight constriction at the junction between the glomerulus and the tubule represents the short neck composed of ciliated cells. These cilia are not very long and do not occlude the lumen as they do in the case

of *Megalops*. Beyond the neck the whole tubule is non-ciliated. Following the neck is the proximal convoluted segment, the wall of which is made up of cells of 12 micra in height with slightly ovoid and almost basally situated nuclei. But towards the distal regions of the tubule the lumen is narrowed



TEXT-FIG. 1. Sections showing the different regions of the renal tubule in *Megalops cyprinoides*. *c*, capsule; *cap*, capillary; *ci*, distal segment; *g*, glomerulus; *n*, neck segment; *pcs*, proximal convoluted segment,; *tu*, tubules obtained by maceration.

down to about 5 micra and the cells also become low cuboidal with centrally placed round nuclei. The staining property of the cells from the two different regions do not show any striking difference. The total length of each tubule is invariably far greater than those in the kidney of *Megalops*, often exceeding 1·5 mm. in length while those in *Megalops* are usually around 1·0 mm. in

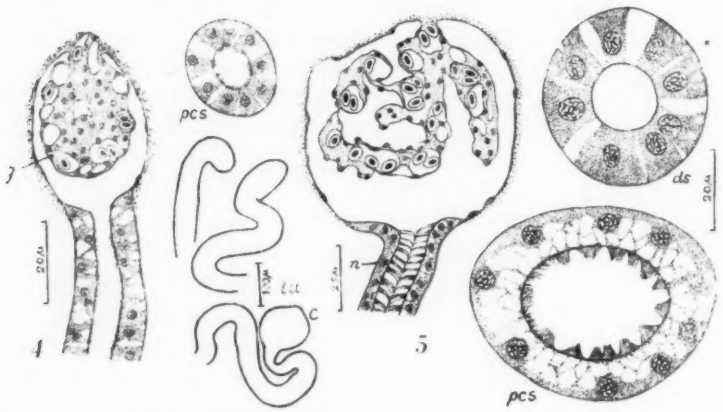


TEXT-FIGS. 2 and 3. Renal tubules of *Chanos chanos* and *Chirocentrus dorab*, respectively *ic*, initial collecting duct (other lettering the same as in the previous figure).

length. However, there is similarity in the structure of the tubules in the two species and both possess a distal region in the tubule distinguishable from the proximal segment.

3. *Chirocentrus dorab* (Fig. 3).—This is one of the species in the collection which shows a fairly small glomerulus, measuring only 30 micra in diameter. The glomerular tuft also indicates a correspondingly poor degree of branching. There is a short neck region of nearly 50 micra in length and of 6 micra in the width of the lumen where the cells are cuboidal but devoid of cilia. The cytoplasm of the cells stain only light with iron hæmatoxylin while the nuclei stain dark and have plenty of chromatin granules. At the end of this convoluted region is a much shorter segment with a very narrow lumen while the cells become tall and rather irregularly placed nuclei. The structure of this terminal portion of the tubule differs very much from that of the distal segment seen in either *Megalops* or *Chanos*, and resembles the initial portions of the collecting duct rather than a part of the uriniferous tubule itself.

4. *Sardinella longiceps* (Fig. 4).—The kidney itself is very narrow and slender and is composed of fewer tubules and little of lymphoid tissue than the kidney of many other species of teleosts given in this account. But these uriniferous tubules are comparatively much longer and the kidney itself is well vascularised. The glomerulus, although with only a diameter of 39 micra, has a branched capillary tuft filling the capsule. In the initial portions immediately following the glomerulus for about 20 micra the cells have scanty cytoplasm and indistinct cell boundaries. The rest of the tubule has



TEXT-FIGS. 4 and 5. Renal tubules of *Sardinella longiceps* and *Muræna favigenea*, respectively. Letterings are the same as in previous figures.

more or less the same internal diameter of 12 micra and the cells have uniformly staining cytoplasm, round nuclei and definite cell walls. There is a fine ciliated border throughout the whole tubule until it joins the collecting duct.

5. *Muræna favigenea* (Fig. 5).—This species shows the largest glomerulus in the present series and measures on an average 138 micra in diameter with a well-developed capillary tuft within the capsule. A short neck of about 130 micra long is seen characterised by the presence of long cilia projecting into the lumen which has a diameter of 14 micra. The cells of this region have almost clear cytoplasm staining light with iron hæmatoxylin and ovoid nuclei. The next region or the proximal segment is the longest in the tubule. Here too the cells are ciliated but apart from the very wide lumen of about 25 micra this segment can also be distinguished owing to the nature of the cells in which are found rather large round nuclei and the cytoplasm is granular and accumulated mostly around the nuclei leaving vacuoles within the cells. Whether these are artefacts or not cannot be certain from this study, and no cell inclusions have been observed in any of these vacuoles. Following the proximal segment there is reduction in the lumen to about 18 micra in the distal segment where the cells are without cilia, the cell walls more distinct and no vacuoles are present. Their nuclei is nearly ovoidal and centrally situated while the cytoplasm tends to stain more deeply along the outer ends of the cells. Another histologically different segment follows the distal segment, the cells of which seem to suggest a structure more like the initial regions of the collecting duct where more than one tubule open before forming the common collecting duct.

Maceration preparations of the kidney have revealed the presence of a number of aglomerular tubules besides the normal glomerular tubules. These aglomerular tubules shown in the figure have a blind ending and their initial regions correspond to the proximal convoluted segment of the glomerular tubules. Thus, excepting for the absence of the golmerulus and the neck these aglomerular tubules do not show any difference in structure from the normal type of tubules and both types open into the same common collecting duct. According to Edwards (1930) the kidney in *Muræna helena* also consists of both glomerular and aglomerular tubules but he has distinguished two ciliated segments following the neck region which have not been made out in the present species.

6. *Plotosus arab.*—The highly branching nature of the capillary tuft is clearly seen in the glomerulus which itself is large and measures 67 micra on an average. The neck is very short and is only about 50 micra long with a lumen of 14 micra and the cells are cuboidal possessing long cilia. The

rest of the tubule is non-ciliated. The proximal segment shows a much wider lumen of nearly 22 micra, the cells in this segment do not have distinct cell walls and the cytoplasm is slightly granular with vacuolations containing darkly staining granules. Their nuclei are round and have single nucleolus. A narrower segment with a lumen of 14 micra, also non-ciliated, follows the proximal region. Here the cells have a more uniform staining cytoplasm, without vacuoles and with distinct cell walls, and the nuclei tend to be nearer the lumen end of the cells. This last region of the tubule seems comparable to the distal convoluted segment.

7. *Saurida tumbil.*—The kidney is rich in lymphoid tissue and only a few tubules are seen in a section. The glomerulus measures 56 micra and the tubules appear to be less convoluted than in many other species. No marked change in the histology of the tubule could be made out along the length of the tubules so as to differentiate them into distinct regions except that the lumen immediately following the glomerulus is ciliated while in the remaining portions there are no cilia. The cells take a uniform stain with iron hæmatoxylin and possess round deeply staining nuclei. Thus, the whole tubule seems to be composed of only a proximal segment and there does not seem to be any indication of having a distal segment.

8. *Cypselurus oligolepis.*—There is relatively little of lymphoid tissue in the kidney which in sections appear to be composed almost entirely of tubules. The glomerulus measures an on average 45 micra and the capillary branching is not very conspicuous. The neck portion has usually an internal diameter of 17 micra and tall distinct cells of 18 micra high. Their cytoplasm stains well and is non-vacuolated, the nuclei are ovoid and situated slightly near the lumen end of the cells. Throughout this proximal segment the lumen is lined by fine cilia whereas in the succeeding region which is short cilia are absent, the cells are without clear cell walls, the cytoplasmic contents seem meagre and the nuclei are centrally located. The structure of this region recalls that of the initial collecting duct leading to the common urinary duct.

9. *Mugil troscheli.*—The glomeruli are of medium size measuring 45 micra on an average with a branching capillary tuft almost filling the capsule. The resemblance between the glomerulus of this species and that of *Chanos* is striking in many respects. The junction of the glomerulus and the tubule is constricted but excepting for this a separate neck region can hardly be distinguished from the tubule proper which has a more or less uniform diameter and structure throughout its entire length. It has a lumen of 8 micra and the proximal region has a ciliated border with cuboidal cells which stain wel

with iron hæmatoxylin and have round nuclei with plenty of chromatin. But towards the distal end there is a long segment with almost the same kind of cells excepting for the absence of the ciliated border. This long non-ciliated region of the tubule is the distal segment.

10. *Sphyræna jello.*—The diameter of the glomerulus is about 40 micra on an average and the capillary tuft shows very poor branching. Starting with a narrow lumen of only 6 micra at the neck the tubule widens in the proximal segment to about 17 micra. But the structure of the cells throughout the tubule seems the same. They are finely ciliated and have a basal round nucleus. Darkly staining granules of varying sizes are seen in many of the cells while some of the cells show vacuoles in them. Towards the succeeding regions the cytoplasm of the cells are only faintly granular and the nuclei are round and deeply staining with plenty of chromatin granules. The lumen in these regions has a narrower diameter of only 12 micra when it opens into the collecting tubule and this region may be distinguished as a distal segment, although it remains somewhat doubtful.

11. *Polynemus tetradactylus.*—The kidney is rich in lymphoid tissue and in sections somewhat resembles the kidney of *Saurida*. The glomerus is large having an average diameter of 65 micra with a well branching capillary tuft. The tubules on leaving the glomerulus has a diameter of 5–7 micra for a very short length of about 20 micra which region represents the neck where the cells are cuboidal, ciliated and have round deeply staining nuclei. This is followed by a long and coiled proximal segment distinguished by a wider lumen nearly four times that of the neck. The cells measure 8 micra in height with finely granular cytoplasm towards their base while at their end near the lumen the cytoplasm is scarce. The major portion is of this type and a separate distal segment seems to be wanting in these tubules.

12. *Cybium guttatum.*—The lymphoid tissue among the tubules is prominent though in much less quantity than in the kidney of *Polynemus* and the glomeruli are also much smaller measuring only 38 micra on an average. Although the size of the glomerulus and its capillary tuft is small the extent of branching of these capillaries is far more when compared to similar sized glomeruli in certain other species. At the junction of the glomerulus and the tubule the diameter of the lumen is only about 5 micra. However, this region is very short extending to only about 30 micra in length and the lumen widens to 9–12 micra in the proximal convoluted segment. The entire tubule is made up of the same type of cuboidal cells of 8 micra in height with uniformly light staining cytoplasm and round nuclei and cilia are absent. The tubule straight opens into a common urinary duct which, unlike the

uriniferous tubules, is composed of tall ciliated cells. Often, lightly staining accumulations have been observed within the lumen of the collecting duct which is unique and whose significance is not known.

13. *Trichiurus haumela.*—The glomeruli are small as in the case of *Cybium*, with an average diameter of 39 micra. But in a single section of the kidney several glomeruli can be seen suggesting the presence of a relatively large number in the whole kidney as compared with many other species described so far. The capillary tuft within the capsule shows a very poor extent of branching leaving a large vacant space within the capsule. A short neck of 7 micra in lumen and composed of ciliated cells having uniformly staining cytoplasm and round nuclei is followed by the proximal convoluted segment. This section has a diameter varying between 10–14 micra and is more or less uniform in its structure throughout its length. The cells are taller than those on the neck region, their cytoplasm is slightly granular and the nuclei are comparatively larger. The whole lumen of the proximal segment is ciliated and communicated directly with the initial regions of the collecting duct without showing a separate distal segment.

14. *Caranx affinis.*—Considering the small size of the corpuscles, the capillary branching may be regarded as moderate as compared to corpuscles of similar size of other species. The neck region of the tubule is extremely narrow with a lumen of only 3–4 micra and extends for about 15 micra in length. The cytoplasm of the cells as well as their nuclei stain well. There is a fine ciliated border in the lumen of the entire tubule. The proximal convoluted region has a comparatively wider lumen of 4 micra where the cells have a faintly granular cytoplasm and the nuclei are round with well staining chromatin granules. This region eventually joins the collecting duct and no segment corresponding to a distal region is noticed.

15. *Therapon puta.*—The kidney has very little lymphoid tissue. Glomeruli are frequently seen in sections of the kidney and are small measuring on an average only 30 micra. The glomerular tuft itself consists of moderate branching capillaries. The whole proximal convoluted segment has a uniform diameter of 10 micra composed of cells having nuclei with a darkly staining nucleolus and chromatin granules. No separate distal segment in the tubule could be made out in this sepcies.

16. *Teuthis marmoratus.*—The kidney is a thin, flat organ composed largely of uriniferous tubules and little of lymphoid tissue. The glomerulus has an average diameter of 49 micra and their number per section is considerably less than in *Therapon*. However, a well branching capillary tuft may be observed within the capsule. There is a ciliated neck region with

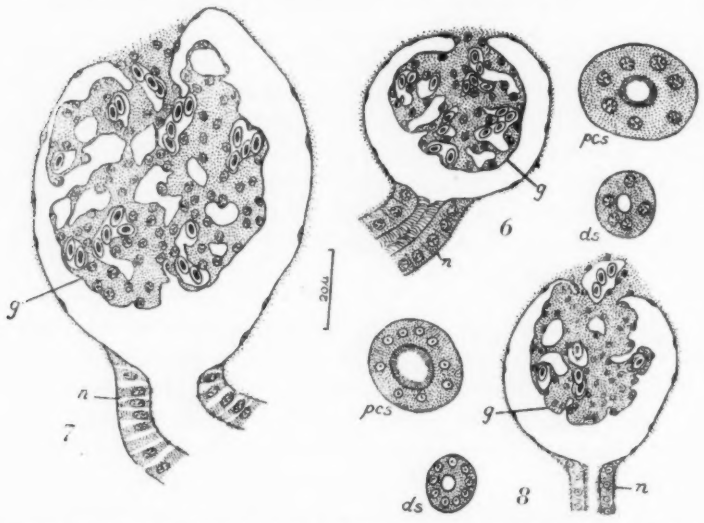
a narrow lumen of 4 micra extending for about 100 micra in length where the cells are nearly cuboidal with indistinct cell walls. The remaining major portion of the tubule is the proximal segment with a lumen diameter of 13 micra and the cells are also low cuboidal. Following this is the short distal segment with about the same internal diameter but whose cells are relatively taller of about 15 micra with round nuclei situated in the middle or sometimes towards the base of the cells. The cytoplasm is granular and in many cells large empty vacuoles may be seen. Throughout the proximal and distal regions of the tubule a fine ciliated border is present.

17. *Platycephalus scaber*.—The lymphoid tissue is comparatively richer than in the previous two species, the glomeruli are much larger having a diameter of 82 micra and have highly branching capillary tuft. The tubules do not show any constriction at the junction of the glomerulus but have nearly a uniform lumen of 12–15 micra for most of their length. The cytoplasm of the cells appear denser around the nuclei and cilia are also absent. Thus, only the proximal convoluted segment exists in these tubules and no distal segment could be distinguished.

18. *Etroplus maculatus* (Fig. 6).—The glomeruli are of medium size measuring on an average 47 micra. The capillary branching is very distinct in the glomerular tuft which occupies most of the available space within the capsule. Thus, although the glomeruli do not attain a size often given for many of the freshwater species these show a good development and offer a fair amount of glomerular surface. The tubule starts with a ciliated neck of 6–7 micra while in the proximal convoluted segment the lumen is slightly wider and the cilia are shorter. Here the cells have an average height of 8 micra with well staining round nuclei and uniformly staining cytoplasm. Following the proximal segment is a short narrow region whose cells are much shorter and also characterised by the absence of the ciliated border. This distal segment compares with that in the kidney of *Chanos*.

19. *Boleophthalmus boddærti* (Fig. 7).—The glomeruli measure on an average of 97 micra. The capsule is made up of thin epithelium and the well branching capillaries of the glomerular tuft is evident in sections. The tubule does not show any distinct neck and its internal diameter is more or less uniform throughout with a diameter of 20 micra and a fine ciliated border. The cells have a deeply staining nuclei while the cells themselves take only a light stain with iron hæmatoxylin. But towards the distal portions of the tubule the cells tend to become slightly taller and stain deeper than those of the proximal region.

20. *Tetradon lunaris* (Fig. 8).—The kidney is soft and composed of a large amount of lymphoid tissue. The glomeruli are not very numerous and do not show any high degree of capillary branching, measuring on an average 44 micra. Both the neck and the proximal segment is ciliated but in the latter the lumen is nearly three times wider than that of the neck, and again in the distal segment the lumen is reduced to less than 5 micra and where cilia are absent. The cells of the proximal region are slightly taller but their nuclei have very little chromatin and stain less intensely.



TEXT-FIGS. 6, 7 and 8. Renal tubules of *Etroplus maculatus*, *Boleophthalmus boddærti* and *Tetradon lunaris*, respectively. Letterings as in previous figures.

DISCUSSION

The renal units in all the species of teleosts described in this account conform to the basic pattern of a functional glomerulus connected to a tubule, the length of which may vary even within the same kidney as pointed out by Marshall (1934). Measurements of the glomerular size of 43 species of fish were made and when arbitrarily classified according to the criteria developed by Marshall and Smith (1930) it is seen that all the species resolve themselves into three groups as given in table. None of these could be assigned to what they regard as Group IV, with very few or no functional glomeruli. The situation becomes somewhat apparent if we take into consideration the habitat of the species and their distribution within the coastal waters. Some of the species are pelagic forms such as *Chirocentrus*, *Sardi-*

nella or *Cybium* inhabiting the relatively more saline zones while many others are confined to areas nearer the coast. Our coastal waters where the fishing activity is largely concentrated are well known for their periodic fluctuations in salinity depending on seasons and especially during the monsoons when, owing to heavy drainage from the land and the river systems, a considerable reduction in salinity occurs along the coastal belt. Therefore, several of the species inhabiting this coastal strip seem to have necessarily developed varying degrees of adaptational powers to lower salinities. It may be seen in the table that a greater number of the more euryhaline forms with better developed glomeruli fall under Group I and II while those under Group III comprise largely of the more stenohaline species. Although the range in the size of the glomeruli does not compare with that given in the extensive data furnished by Marshall and Smith (*loc. cit.*), the general tendency for the more euryhaline species to classify themselves under the same group is obvious from the table.

It may be pointed out here that the differentiation into the various regions of the renal tubules as given in this account is based only on histologic characteristics. In order to understand their true significance or their functional differentiation corresponding to these histologic changes, we need critical experimental evidence which is wanting in the case of our teleostean fishes. However, we can find a general agreement with some of the well-known facts that the more euryhaline species possess a well developed glomerulus indicative of an efficient filteration mechanism, together with the presence of a distal segment in their tubule. This is a common feature with many of the freshwater species where the function of the distal segment is believed to be reabsorption of salts from the glomerular filtrate leaving a relatively dilute urine for excretion. On the other hand, in true marine fishes which never invade waters of lower salinities and consequently have no excess water to get rid of their body the distal segment in the renal tubule is said to be completely absent (Smith, 1953). This general pattern of structure is by no means rigid and exceptions occur within the three groups as given in the table, and the reasons for the variations in individual instances cannot be adequately explained in this state of our knowledge of the excretory and adaptational physiology of the species. From field observations and experience in practical fish culture we do know that many species can withstand surprisingly low or high levels of salt concentrations in their environment although reliable data on the limits of tolerance levels for many of our species of fish are lacking at present. Among those marine species which can tolerate very low salt concentrations *Chanos chanos* and *Mugil* spp. are of special importance owing to their utility for fish culture. Several

TABLE

Sl. No.	Species[1]	Diameter of glomerulus	Habitat[2]	Distal segment[3]
	GROUP I			
1	*Megalops cyprinoides* (1)	64	M & E	+
2	*Muræna favigenea* (5) ..	138	M	+
3	*Murenesox cinereus* ..	60	M	
4	*Arius jella* ..	58	E	
5	*A. maculatus* ..	65	E	
6	*A. dussumieri* ..	72	E	
7	*Plotosus arab* ..	67	M & E	+
8	*Macrones vittatus* ..	42	M & E	
9	*Polynemus tetradactylus* ..	65	M	–
10	*Platycephalus scaber* ..	82	M & E	–
11	*Etroplus maculatus* (6) ..	47	F & E	+
12	*E. suratensis* ..	47	F & E	
13	*Boleophthalmus boddærti* (7) ..	97	E	+
	GROUP II			
14	*Chanos chanos* (2) ..	47	M & E	+
15	*Engraulis malabaricus* ..	42	M	
16	*E. mystax* ..	37	M	
17	*Dorosoma chacunda* ..	44	M & E	
18	*Saurida tumbil* ..	54	M	–
19	*Tylosurus strongylurus* ..	42	M	
20	*Hemirhamphus georgii* ..	37	M	
21	*Cypselurus oligolepis* ..	45	M	–
22	*Cynoglossus* sp. ..	57	M	
23	*Mugil troscheli* ..	45	M & E	+
24	*Sphyræna jello* ..	40	M	(?)
25	*Mene maculata* ..	43	M	
26	*Gerres abbreviatus* ..	40	M	
27	*Teuthis marmoratus* ..	49	M	+ (?)
28	*Psammoperca waigiensis* ..	56	M	
29	*Therapon puta* ..	52	M & E	–
30	*Tetradon lunaris* (8) ..	44	M & E	+

TABLE I—*Contd.*

Sl. No.	Species[1]	Diameter of glomerulus	Habitat[2]	Distal segment[3]
	GROUP III			
31	*Chirocentrus dorab* (3) ..	30	M	—
32	*Pellona brachysoma* ..	40	M	
33	*Sardinella longiceps* (4) ..	39	M	—
34	*Opisthopterus tartoor* ..	38	M	
35	*Cybium guttatum* ..	38	M	—
36	*Trichiurus haumela* ..	39	M	—
37	*Caranx affinis* ..	38	M	—
38	*Chorinemus lysan* ..	37	M	
39	*Lactarius lactarius* ..	38	M	
40	*Leiognathus splendens* ..	39	M	
41	*Sciæna* sp. ..	37	M	
42	*Otolithus ruber* ..	30	M	
43	*Ephippus orbis* ..	43	M	

[1] The numbers in brackets against species refer to the corresponding figure and description in text.

[2] Gives the usual habitat of the species as M = marine, E = estuarine and F = freshwater.

[3] A *plus* sign (+) indicates presence of the distal segment, a *minus* sign (−) shows its absence while a (?) mark denotes doubtful cases.

other species also show the same behaviour. Many species of fish are capable of gradual acclimatisation to either lower or higher salinities than their normal environment and there are several records in literature of successful acclimatisation. Recently the freshwater species *Etroplus maculatus* has been shown to be able to withstand a salt content up to 32·6 parts per thousand (Rao, 1958). Similarly the freshwater fish *Tilapia mossambica* is another example which, on account of its ability to withstand even hypersalinities and successful establishment in such media, are being experimented upon for rearing in marine fish farm ponds (unpublished work of the author). A study of the renal structure of these species would seem particularly interesting in view of such adaptational powers.

While discussing the structure of the renal tubules and the problem of adaptation in teleosts to fresh and salt water, Grafflin (1937 *b*) has rightly pointed out that the kidney is not the limiting factor for survival of the individual in media of different salt concentrations. Ample evidence exists in

literature to show the presence of extrarenal powers of salt regulation in teleosts. Besides as Smith (1932) has remarked, " Like many other evolutionary specialisations the reduction of glomeruli probably proceeds slowly and more detailed palæntological history than is now available may be required to interpret the status of any one family, genus or species". It thus seems that the microscopical structure of the renal unit alone throws little light on the remarkable physiological operations carried out by the kidney but can greatly supplement experimental observations.

The object of this preliminary account, as mentioned earlier in the introduction, is mainly to provide some essential background information on the renal elements in some of the more common teleosts of our coast. However, based on these observations it seems possible to discuss briefly some of the essential lines on which further work seems fruitful. As the marine fishes show a wide range in glomerular development, the need for a more precise quantitative estimation of the glomerular number in relation to body size is felt as this gives a more reliable indication of the efficiency of their osmoregulatory mechanism. Nash (1931) had taken " *the volume* of glomeruli (assumed to be a measure of the filtration surface) per unit of *body surface area* " as the basis for comparison by which he found a sharp distinction between the freshwater and the marine teleosts. However, he had pointed out that the average size of a few glomeruli in the kidney of one individual of a species cannot be considered as an accurate average size for all the glomeruli of that one kidney, much less for the species as a whole. Rytand (1935) working on the mammalian kidney observed a close relationship between the total glomerular volume both to the kidney weight and to the body weight in mammals of different size. Gunter (1945) in his studies on the distribution of fishes along the coast of Texas observed seasonal movements of many of the relatively smaller members of the species penetrating brackish waters more persistently than the larger ones. This is also true of many of our fishes which generally show better adaptational powers in their younger stages and consequently enter tidal creeks and estuaries. This among other things, might suggest some kind of change in their excretory mechanism during the different phases in their growth which has to be carefully assessed on a quantitative basis. The assigning of definite physiological functions to the different parts of the renal tubule in the normal fish without interference by operational procedures is mainly due to the anatomical and experimental studies of Grafflin (1937 *b*) and Marshall and Grafflin (1942) and more recently of Forster (1953) and similar detailed work on some of the important species of culturable fish would be of great interest. In this connection we are also unable to overlook the fact that the question of

osmotic regulation is intimately tied up with the physiological functions of certain other organs of the body, especially some of the endocrine glands which play an important role in initiating changes which are necessary for the successful penetration of the fish into new environment as has been pointed out by Black (1951). Similarly, the significance of certain essential salts such as calcium in the survival of fish is also a vital factor as indicated by Breder (1933) and Panikkar *et al.* (1953). Thus, there are many important gaps in our knowledge which have to be understood before the significance of structural and functional morphology of the renal units in different species of fish can be properly interpreted.

Summary

The general histology of the renal units of 20 selected species of teleosts has been given. Besides, an analysis of the glomerular size in a total of 43 species of fish shows good agreement with some of the well-known theories of glomerular development in relation to habitat. This preliminary study has helped to bring out some of the essential lines on which reliable data on the renal physiology of some of the euryhaline fishes have to be obtained and these are briefly discussed.

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TWO SPECIES OF WOOD-BORING PHOLADS OF MADRAS*

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The two pholads described in this paper belong to the subgenus *Martesia* and have been recorded from all over the world (Sowerby, 1824; Gray, 1840, 1875; Tryon, 1862; Verrill and Bush, 1890; Bartsch and Rehder, 1945; Turner, 1955). In the Indo-Pacific area, particularly in India, only *Martesia* (*Martesia*) *striata* has been collected and studied from different places on the Indian coast (Beeson, 1936; Erlanson, 1936; Gravely, 1941; Ganapathi and Nagabhushanam, 1957; Pampapathi Rao *et al.*, 1957). The occurrence of the second species of wood-boring pholad *M* (*M*) *fragilis* in the Indo-Pacific area was first reported from Madras by Srinivasan and Daniel (1956). In view of the fact that these two species cause extensive damage to wooden fishing craft and wooden harbour structures, full descriptions of these two forms were considered worthwhile. The diagnostic characters of these two species and their allies are also described from live and preserved specimens.

I. *Martesia* (*Martesia*) *striata* Linné, 1758

Pholas striata Linné, 1758, *Systema naturæ*, **10**, 69.

Pholas corticaria Gray, Sowerby, 1849, *Thesaurus conchyliorum*, **2**, (10), 495.

Martesia striata Linné, Tryon, 1862, *Proc. Acad. Nat. Sci., Philadelphia*, p. 92.

Martesia curta Sowerby, Dall, 1909, *Proc. Uni. St. Nat. Museum*, **37**, 161.

Penitella xylophaga 'Valenciennes', Lamy, 1921, *Bull. Mus. d'Histoire Naturelle, Paris*, p. 27.

Martesia (*Diploplax*) *americana*; *Martesia* (*Diploplax*) *funisicola* Bartsch and Rehder, 1945, *Smith Misc. Coll.*, **104**, 13 and 14 (Young).

Martesia intercalata 'Carpenter', Hertlein and Strong, 1950, *Zoologica*, **35**, (19), 250.

* Formed part of a thesis approved for the M.Sc Degree of the Madras University.

Martesia revicola Lamy 1925, *Jour. de Conchyl*, **69**, 19.

Martesia striata Satyamoorthy, 1956, *Bull. Mad. Govt. Mus.* (*N.S.*), **1**, 168.

Martesia striata, Turner, 1955, *Johnsonia Mus. Comp. Zool., Harvard University*, **3**, (34), 65.

Locality.—2,500 specimens were collected from piles in Madras Harbour and also from catamarans at Madras, Porto Novo, Tuticorin and Kayankulam.

Shell.—The shell is pyriform (Fig. 1 *a*) measuring 9–11 mm. vertically at the broad inflated anterior part and 18–22 mm. from the anterior margin to the tip of the posterior narrow part.

The two valves are articulated by an interlocking of their hinge teeth and by a tuberosity on the right valve. The axial edge along which the valves are joined extends slightly below the umbonal elevation or beak. Extending from the edge upwards and forwards is a small shell formation called "the flange" (Figs. 1 *b*, 1 *c*, F) which lies between the hinge teeth and the articular process and serves for the attachment of the anterior adductor. On the outside of the shell where anterior edge curves backwards to meet the inflated umbonal area a deep pit occurs (Fig. 1 *b*, FP).

Externally each valve is divided into two regions, the anterior and posterior by an oblique line, *viz*., the umbonal ventral sulcus (Fig. 1 *a*, UVS). This sulcus starts from the mesoplax (Fig. 1 *a*), and runs down to the ventral side as far as the hypoplax (*vide infra*). In a specimen of about 24 mm. length the anterior portion forms about 8 mm. and has 18–20 denticulate ridges making its surface rough and rasp-like. Antero-ventrally, the front part of the shell is smooth and is distinguished as the "callum" (Fig. 1 *a*, CA). The callum of the two valves meet in the middle and thus close the pedal gape present in younger forms (Fig. 1 *e*) in which the foot is well developed. Internally the sulcus is marked by a ridge (Fig. 1 *c*). Impressions of a large posterior adductor, a smaller anterior adductor and the accessory ventral adductor are seen. The pallial sinus is broad and deep. The apophysis (AP) is narrow, spatulate and extends from the umbone towards the ventral side.

The accessory plates.—Three accessory plates are found namely (1) Mesoplax, (2) Metaplax and (3) Hypoplax. The mesoplax and metaplax are median and dorsal to the valve while the hypoplax is ventral (Fig. 1 *a*).

During the growth of the pholad, the mesoplax makes its appearance, when the young are 3 mm. long, as a thick lip over the antero-dorsal area

of the shell (Figs. 1 *e*, *f*, *g*). Later a shield-shaped plate is added extending backwards towards the umbo. In the adult (Figs. 1 *a* and 1 *d*, M) the mesoplax is a fused composite plate in front of the umbonal area. However, the two components can be made out from the ridges borne by them. The shield-shaped plate is flatter and is marked by irregular ridges externally and appears longer than the other plate. The other piece formed earlier appears larger from the inside and is marked by concentric ridges proceeding from a median notch on either side of the median line. The fused plate appears inflated (Fig. 1 *d*).

The metaplax (Figs. 1 *a*, ME; 1 *b*) is a large slender plate whose broad front-end is tucked under the mesoplax and whose narrow tapering hind tip extends to the posterior margin of the valves uniting them.

The hypoplax (Figs. 1 *a*, HY) is a narrow slender plate extending from the callum as far behind as the ventral margins and unites the valves ventrally. In the young forms of about 3 mm. length the metaplax and the hypoplax plates are not found and therefore the valves can be moved apart.

II. *Martesia* (*Martesia*) *fragilis* VERRILL AND BUSH, 1890

Martesia (*Martesiella*) *fragilis* Verrill and Bush, 1890, *Proc. Unit. St. Nat. Museum*, **20,** (1139), 777.

Pholadidea (*Penitella*) *minuscula* Dall, 1909, *Bull. Mus. Comp. Zool.*, **43,** 425.

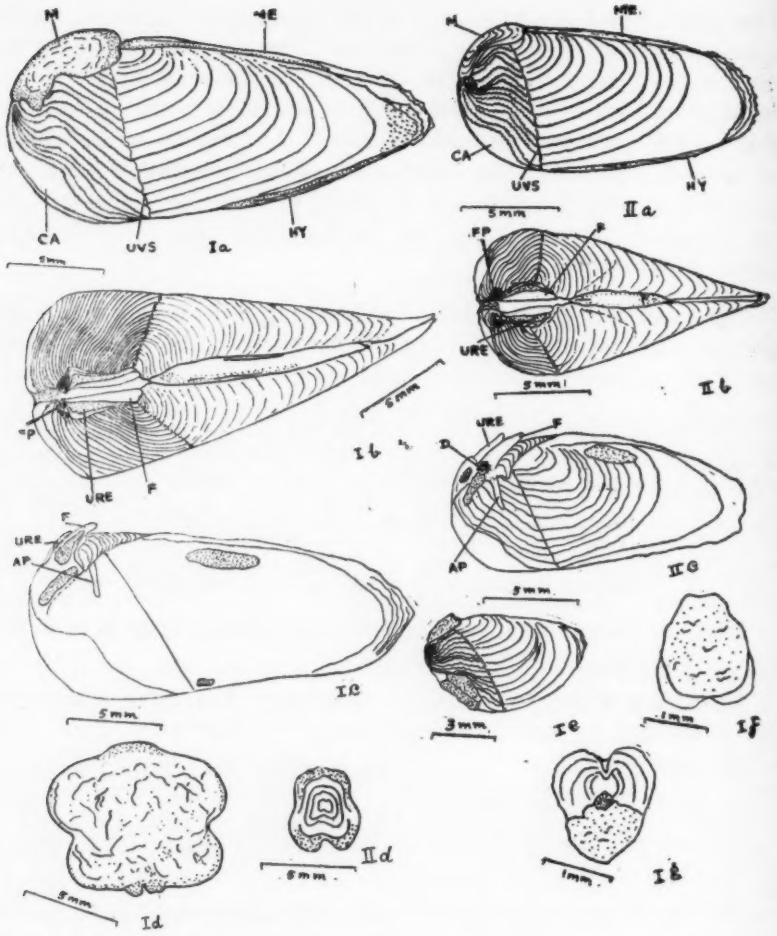
Martesia (*Diploplax*) *exquisita* Bartsch and Rehder, 1945, *Smith Misc. Coll.*, **104**, (11), 10.

Martesia (*Diploplax*) *bahamensis* Bartsch and Rehder, 1945, *Ibid.*, **104** (11), 11.

Martesia (*Martesia*) *fragilis* Turner, 1954, *Johnsonia* No. 34, p. 111.

Martesia (*Martesia*) *fragilis* Srinivasan and Daniel, 1956, *Curr. Sci.*, **25**, 59.

Occurrence.—More than 1,500 specimens were collected at Porto Novo and Madras from floating pieces of soft timber like *Myristica fragrans* and *Mangifera indica.* These soft shelled forms do not occur in the Madras Harbour where harder timber *Tectona grandis*, *Shorea robusta* are used. This form was first recorded only four years ago by Turner (1955) from the Western Atlantic. The present account is the first description of the form occurring in the Indo-Pacific area.



TEXT-FIG. 1. *Martesia* (*Martesia*) *striata*.
(*a*) Outer view of the shell.
(*b*) Dorsal view of the shell (Mesoplax removed).
(*c*) Inner view of the right shell valve.
(*d*) Outer view of the Mesoplax.
(*e*) Outer view of the young shell of *Martesia* (*Martesia*) *striata*.
(*f*) Dorsal view of the Mesoplax of the young shell of *M. striata*.
(*g*) Ventral view of the Mesoplax of the young shell of *M. striata*.

TEXT-FIG. 2. *Martesia* (*Martesia*) *fragilis*.
(*a*) Outer view of the shell.
(*b*) Dorsal view of the shell (Mesoplax removed).
(*c*) Inner view of the right shell valve.
(*d*) Outer view of the Mesoplax.

AP, Apophysis; CA, Callum; D, Denticles; F, Flange; FP, Funnel-shaped pit; HY, Hypoplax; M, Mesoplax; ME, Metaplax,; URE, Umbonal reflection; UVS, Umbonal ventral sulcus.

The shell (Figs. 2 *a*, *b*).—The two valves are very fragile and measures 6–17 mm. in length and 4–12 mm. in breadth. The specimens collected from timber samples vary widely in the shape of the shells, some being pyriform and others being elongately oval and some others being intermediate between. Besides this variability in shape, the shell of the present form can be distinguished from *M. striata* by the following features: The umbonal reflection (Fig. 2 *b*, URE) is twice as thick as in *M. striata*. The flange (Figs. 2 *b*, *c*, F) which gives attachment to the anterior adductor is elongately triangular whereas it is sickle-shaped in *M. striata*. The anterior part of the shell forms two-thirds of the posterior, whereas in *M. striata* it is only half. Hinge teeth are absent in the present form but there are a few denticles (Fig. 2 *c*) on each valve in front of the umbonal articulations which help in interlocking the valves together. The shell valves of both species are similar in the absence of a condyle present at the lower end of the sulcus of the wood-boring xylophaginæ.

Accessory plates.—The mesoplax is either oval or cordate, whereas in *M. striata*, this plate is never cordate though circular plates are met with. They are also fragile in the present species and differ slightly in form (Figs. 2 *a*, *b* and *d*, M). Dorsally the sculpturing is concentric whereas in *M. striata* it is irregular. The metaplax is larger proportionate to the length of the whole shell and is lanceolate in outline (Figs. 2 *a*, *b*, ME) whereas in *M. striata* it is relatively shorter and narrower. The hypoplax is also lanceolate in *M. fragilis* (Fig. 2 *a*, HY) whereas it is uniformly narrow in *M. striata*. In a specimen of 9 mm. length the metaplax and hypoplax measure 3·5 mm. and 2· 5 mm. respectively.

Taxonomic remarks.—The earliest authors who founded the genus *Martesia* (Leach, 1824) and described it (Sowerby, 1824) had taken into account so few of the features of *Martesia* that by 1945 Bartsch and Rehder found it necessary to establish 4 subgenera; *Martesia* (*Martesia*), *Martesia*, (*Diploplax*), *Martesia* (*Diplothyra*) and *Martesia* (*Particoma*). In 1955, however, Turner found that the young forms do not have the callum which is a feature of adult growth and therefore the species assigned to *Martesia* (*Diploplax*) because of the absence of the callum had to be transferred to *Martesia* (*Martesia*). Turner also found that the forms included in the subgenus *Diplothyra* are characterised by the extension of the callum along the dorsal margin, the mesoplax being rectangular and both the metaplax and the hypoplax being bifid posteriorly. These features are as important as those distinguishing the genus *Martesia* itself. Therefore she gave *Diplothyra* the status of a separate genus. This left only two subgenera namely *Martesia*

(*Martesia*) and *Martesia* (*Particoma*) in the genus. The only species of *Martesia* (*Particoma*) namely *Martesia* (*Particoma*) *cuneiformis* has not been recorded from India.

The present author reared the young ones of the two wood-boring species *M. striata* and *M. fragilis* in the laboratory to the adult condition and found that they grew the callum. This confirms Turner's observation and supports her rejection of *Diploplax* as a subgenus separate from *M.* (*Martesia*).

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ERRATUM

Vol. XLIX, No. 6, page 400, in Table I, second and fourth lines,
for Dactylus/Propus *read* Propus/Dactylus.

STUDIES ON THE PHYTOPLANKTON OF THE WEST COAST OF INDIA

Part I. Quantitative and Qualitative Fluctuation of the Total Phytoplankton Crop, The Zooplankton Crop and Their Interrelationship, with Remarks on the Magnitude of the Standing Crop and Production of Matter and Their Relationship to Fish Landings*

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Received March 13, 1958

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I. INTRODUCTION

IN the temperate and polar waters considerable work has been done on the qualitative and quantitative fluctuation of the phytoplankton as well as on their production and rate of production of other organic matter by them; and, consequently, we have a good knowledge of the fertility of these waters. On the other hand, our knowledge about these aspects of the tropical and sub-tropical zones of the world is very meagre indeed, and all our knowledge about these regions until very recently was derived from the reports of Expeditions (the *Challenger*, *Valdivia*, *Meteor*, *Michæl Sars*, *Carniegie*, *John Murray*, *Dana* and so on) and as has been pointed out with emphasis by Dakin and Colefax (1940) these reports are often misleading as to the true conditions and give one an erroneous picture of the plankton conditions of the warmer regions, mainly because the conclusions are based on casual and discontinuous observations made during off-seasons while passing through the tropical regions to reach the temperate zones at or during the favourable season there.

Further, it is generally held that tropical waters are not so productive as compared with the colder seas. Some of the investigations in recent times have shown, however, that when all facts are taken into consideration,—the increased metabolic rate in the warmer regions, the rate of turnover, and the frequent records of heavy crops of zooplankton in certain areas—tropical and sub-tropical waters also are to be considered productive when compared with the colder regions (Russel, 1934 *b*; Russel and Coleman, 1934; Allen, 1924, 1934 and 1939; Seiwell, 1935 *a*; Gilson, 1937; Dakin and Colefax, 1940; Riley, 1938, 1939, 1944; Riley, Stommel and Bumpus, 1949; Graham, 1941 *a*; and King and Demond, 1953; *cf.* however Steemann Nielsen, 1951 *a*, 1952, 1954). Again, certain areas near the shore in the warmer regions are known to support a heavy crop of plankton such as the west coast of Africa (Hentschel and Wattenberg, 1930) and the west coast of S. America (Gunther,

1936). According to Graham (1941 *a*) plankton production is not a function of lattitude but depends upon presence of "new water" high in nutrient salts. He found greater production of total plankton in the tropics between 20° N. and 11° S. than between 20° and 34° N. in the Pacific.

Data relating to phytoplankton of the warmer seas in particular are very meagre. Marshall (1933) has indicated that production of phytoplankton in the Great Barrier Reef region is not poor when compared with certain temperate regions like the English Channel. Gilson (1937, p. 59) after a study of the northern part of the Indian Ocean, including stations in the Arabian Sea, states that "Whatever the value of comparison, it is probably safe to say that the productivity of the tropical upwelling areas exceeds that of any temperate sea, while the open tropical ocean is by no means as barren as has commonly been supposed, and is indeed comparable in this respect with a region such as the English Channel". Allen (1939) has recorded heavy production of phytoplankton in the Panama region. Kow (1953, p. 41) states that the standing crop in the Singapore Straits is not very much lower than that in the English Channel. There are a few accounts by Japanese workers dealing with the plankton of the tropical regions in the Pacific (Aikawa, 1942; Kitou, 1952; Motoda, 1940 and 1941; Tokioka, 1942 *a*, 1942 *b*; and Haneda, 1942). The results of Haneda and Tokioka show higher volumes of plankton nearer the Equator.

In India there have been several attempts in the past to study the marine plankton of both the east and west coasts of the Peninsula (Hornell and Nayudu, 1923; Menon, 1931; Aiyar, Menon and Menon, 1936; Menon, 1945; Chacko, 1950; Chidambaram and Menon, 1945; George, 1953 *a*; Gonzalves, 1947; Prasad, 1954 *a* and 1956; Ganapati and Murthy, 1955; Ganapati and Rao, 1953; and Ramamurthy, 1953 *b*). All these accounts are of a general nature and the duration of the investigations varies from a few months to about three years only. They are, further, all of a qualitative nature with indications of the peak period of plankton production and of the seasonal cycle (Panikkar, 1950). None of the accounts appear to give a quantitative assessment of the crop of plankton in the sea, least of all phytoplankton, the prime synthesizers of food. Most of the accounts, further, claim to be of a preliminary nature and, therefore, do not allow of any definite conclusions to be drawn.

It may be mentioned here that in India in the past, investigations on the phytoplankton in particular appear to have suffered due to the want of a taxonomical appraisal of the constituents of the flora; this may be true of many other regions also. This has been remedied to some extent by the

account of Subrahmanyan (1946) for the Diatomaceæ, the group which constitutes the bulk of the plankton flora.

The writer studied the phytoplankton from all aspects in the scheme of fisheries research recently initiated in India; and, since 1949, he has been engaged in this work. In the present paper, which forms the first part, an account is given of the seasonal fluctuation of the total quantity of the phytoplankton crop and the main groups constituting the same, the total zooplankton crop, the magnitude of the standing crop compared with other regions, the interrelationship between phyto- and zoo-plankton crops, and the results are discussed with reference to fish landings and also compared with those from other geographical areas. Being the first paper from this country on such an aspect of quantitative study on plankton, some pertinent literature has also been reviewed for comparison and relative assessment of the fertility of the waters. This investigation covers a period of over five years and it was extended to such a long period as it was the desire to report, as far as possible, definite and reliable conclusions in the interests of future applied research. In the second part the physical and chemical factors involved in the production of phytoplankton and the relationship of some of the former to fish landings are dealt with.

II. Physical and Meteorological Features of the West Coast of India

For a clear appreciation of the results presented in the following pages and the ecological relationships, a knowledge of the physical features and meteorological conditions of the area is necessary. Particulars given in this section have been drawn from Admiralty (1950) publication, Hornell and Nayudu (1923), Bristow (1938) and Du Cane *et al.* (1938). The meteorological data were obtained through the courtesy of the Director, Regional Meteorological Centre, Madras.

1. *Physical Features*

The west coast of Peninsular India forms a narrow belt of low land, geologically of laterite formation, lying between the sea and the Western Ghats which extend the whole length of the Peninsula varying in width from 20 to 100 miles inland. There is a large number of short rivers many of which discharge into backwaters of varying breadth occurring parallel to the coast. There are two rivers nearer to the present area of investigation—the Kallai on the south and Elathur on the north, both of which open into the sea (Text-Fig. 3).

The coast-line (Bristow, 1938) north of Calicut up to Mount Delli is fringed with low cliffs alternating with reaches of sand; and denuded headlands and bays hollowed out by the waves on their southward side betray the action of the ocean current. At Calicut and southwards the shore is one unbroken stretch of sand partly thrown up by the waves, partly formed by alluvial deposits brought down by the rivers and backwaters.

2. *Meteorological Features*

(*a*) *Seasons.*—The climate of the west coast of India in particular is dominated by the monsoons and four seasons could be distinguished*:

(1) A wet season (May–June to September): The onset of the south-west monsoon is the beginning of the rainy season for most of the region. It commences in May or early in June, exceptionally later in some years by the end of June, at the southern end of Peninsula and advances northwards striking the coast of Sind (Pakistan) in about three weeks.

(2) A short transition period, comprising the end of September and October, hot, steamy, fairly dry season occurs before the north-east monsoon sets in.

(3) The north-east monsoon normally begins earlier in the north than in the south where it becomes well established by December. It continues until about March in which month it becomes unsteady in the south. During this season, there is practically no rainfall on the west coast.

(4) A hot dry season, March and April, characterised by calm weather with light breezes from north north-west and north-west. In May, conditions again become unsettled indicating the onset of the south-west monsoon.

(*b*) *Wind force.*—The wind force at Calicut in the months prior to the outbreak of the south-west monsoon proper is rather high owing to the high temperature and consequent low pressure over the region. With the outbreak of the monsoon proper, the wind force tends to diminish, rises slightly in October (the transition month to the north-east monsoon), falls again a little but increases steadily from January onwards. The data relating to wind force at Calicut are shown in Table I.

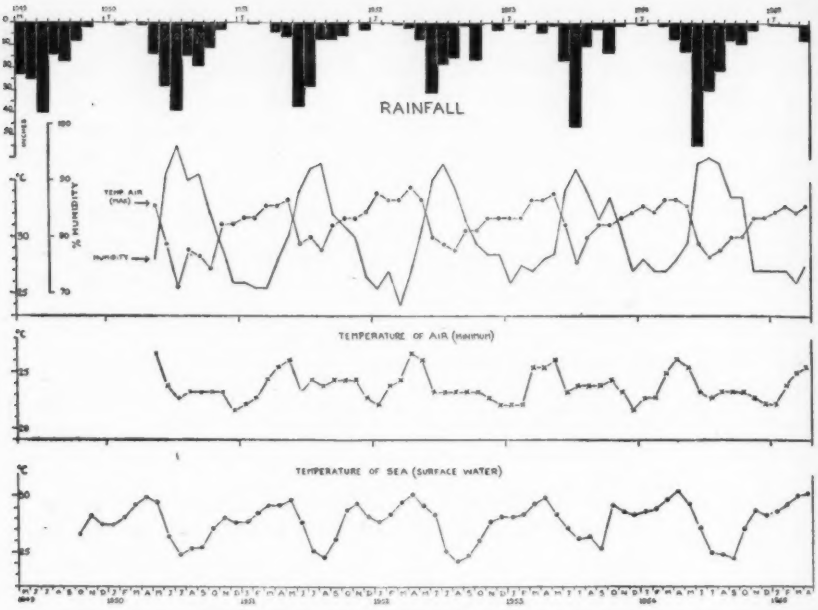
(*c*) *Rainfall.*—The annual rainfall is high over the region varying from 60–130 inches. Most of it falls during the south-west monsoon season. North of 20° rainfall is somewhat less. During the north-east monsoon season, there is very little rainfall over the region.

* More details may be had from Admiralty (1950) publication.

TABLE I

Wind velocity (Miles per hour)

Month	Year	1949–50	1950–51	1951–52	1952–53	1953–54	1954–55	Average of 5 years
May	..	7·7	9·8	8·0	8·0	9·9	8·0	8·6
June	..	5·7	7·5	7·1	5·7	7·1	5·7	6·6
July	..	6·3	5·7	7·0	5·5	6·9	5·5	6·2
August	..	5·6	5·6	5·5	5·3	6·5	6·0	5·8
September	..	5·6	6·2	5·8	5·4	5·7	4·9	5·6
October	..	6·3	6·1	6·0	7·9	5·5	5·5	6·2
November	..	5·4	5·6	5·4	6·2	5·5	5·9	5·6
December	..	6·0	5·7	5·7	5·5	5·7	5·7	5·7
January	..	6·8	6·5	6·1	6·7	6·0	6·5	6·4
February	..	8·7	7·5	7·0	6·9	7·4	8·1	7·6
March	..	8·0	8·0	8·0	8·9	7·6	8·5	8·2
April	..	9·5	9·1	8·2	8·5	8·3	8·3	8·7



TEXT-FIG. 1. Seasonal fluctuation of the temperature of air (maximum and minimum), of water, humidity and total rainfall.

At Calicut, the mean annual rainfall is over 100 inches; over 50% of it falls in the month of June and July of the south-west monsoon season. The total rainfall was 138·4 inches in 1949; it declined during the succeeding years to 92·16 inches in 1952; then rose to 95·7 inches in 1953 and 143·20 inches in 1954 (Text-Fig. 1). The records of rainfall for over several decades (1864–1937) at Cochin (9·58° N., 76·17° E.), south of Calicut, indicate two outstanding peaks, one in 1882 and the second in 1924 and a fairly average succession of medium, high and low intensity during the years in-between (Bristow, 1938). A possibility of a similar cyclical occurrence of rainfall is indicated at Calicut also by the data collected here.

The rainfall at sea about 20 miles from the Malabar and Konkan coasts is believed to be considerably less than on the coast.

(*d*) *Air temperature and humidity*.—The temperature is high over most of the region. The maximum temperature varies from 27·7° C. to 33·3° C. The mean temperature in the hottest month, which is generally just before the onset of the south-west monsoon, is nearly the same over most of the places, but the mean of the coolest month differs considerably.

The temperature of air at Calicut is fairly high (Text-Fig. 1). The highest value for the maximum lies in April (1952–55) or May (1951) and the lowest in July (1953 and 1954) or August (1951 and 1952). The highest value for the minimum temperature also occurs in April or May but the lowest minimum temperature is recorded in December or January. The range between the maximum and minimum is highest in January (average 10·2° C.) and is least during the south-west monsoon months of June and July (average 4·8° C.). A double oscillation in the fluctuation of the temperature of air is evident, clearly expressed when the average daily temperatures are reckoned. The main maximum occurs in April or May and with the onset of the south-west monsoon, the temperature falls till July or August when the difference between the maximum and the minimum is least; then, the temperature rises again and shows a minor peak in October or November (transition months to the north-east monsoon season) after which there is a slight fall in the maximum, but a much greater fall in the minimum value, when the daily range between the two is greatest (December or January), the winter or cold season of the region. (The temperature of the sea surface at Calicut is given and discussed in Part II.)

The *humidity* over the area is very high. It varies from 80–90% during the south-west monsoon months and 65–75% during the north-east monsoon months.

The humidity at Calicut (Text-Fig. 1) during the years of investigation varied from 68% (March 1952) to 94% (June 1954). The mean variation (average of 5 years) at Calicut during the south-west monsoon period is from 79–93%; from May it increases up to July and falls subsequently. During the north-east monsoon season it varies from 72–78%, the lowest value being in March. Humidity increases as rainfall increases and temperature falls. The north-east monsoon is not very active on the western side of the Ghats and there is no appreciable rain after October, the transition month between the monsoons. The humidity, therefore, registers a fall and begins to rise again when the pre-monsoon rains fall in the April following.

From the marine biological aspect, the year may be divided into two periods: (1) the south-west monsoon period from May to September–October and (2) the north-east monsoon period, October–November to April.

III. The Sea Floor, Mud Banks and Currents

1. *Sea Floor*

The Continental shelf (Brown in Du Cane *et al.*, 1938) on the west coast of India extends outward to an average depth of 100 fathoms and at about

20° N. latitude is widest, being over 120 miles and narrows to the south until, off Cape Comorin, it is only 30 miles wide. The 100 fathom contour line has well marked indentations, one north of Cochin and another south of Quilon where the shelf is constricted to about 25 miles or less. The sea floor slopes gradually to a depth of 100 fathoms along the Malabar Coast and makes a steep descent to great depths. Off Mangalore-Cochin section, the sea floor slopes very gradually to about 65 fathoms; beyond, the bottom plunges to the 1,000 fathom line. At about Quilon, south of Calicut, the shelf first slopes to as much as 190 fathoms and then rises to 170 fathoms before falling away steeply in the Continental slope.

The sea bottom, according to Hornell (1910) "from about a mile from shore out to 30 fathoms, from Ponnani northwards to Mangalore, is composed of soft grey mud with small dead shells, etc.". It is believed (Du Cane *et al.*, 1938) that the mud deposits are terrigeneous in character and largely derived from the laterite and alluvial belt of the coast which originated from the disintegration of gneissic rocks of the interior. Mud of both sea bed and mud banks (mentioned below) are thought to be brought down by the present and past rivers.

2. *Mud Banks* (Bristow, 1938; Du Cane *et al.*, 1938)

There are four well-known mud banks off the south-west coast of India, one at Alleppey, one at Cochin, and two at or near Calicut. Mud banks of a less intense nature occur at Quilandy, Tellicherry and a few other places. These banks are formed by fine mud which is in an unconsolidated state and the banks shift from place to place under certain influences which are not clearly understood. Earthquakes and underwater cataclysmic changes and cyclones as also ocean currents have been known to affect the movement of the mud banks at Cochin. Further, the intensity of rainfall is supposed to affect the silting at the approaches in Cochin and the movement of the Narakkal mud bank nearby.

The mud banks at Calicut also are known to shift from place to place and appear or disappear probably depending on the intensity of rainfall and coastal currents. Generally, they become visible during the south-west monsoon season and occur slightly submerged or well visible out of the water at a little distance from the shore. One characteristic feature, when they are present here as well as elsewhere, is that the sea in their environs remains extremely calm even when the roughest weather prevails and the water is very stormy in the surrounding areas. In the region of the mud banks, the water surface has been found to be oily (the oil has similarity to petroleum);

this was believed to effect the calm conditions (Bristow, 1938) ; however, this effect is supposed to be due to the suspension of fine matter increasing the viscosity of the water which helps break up the force of the waves (Keen in Du Cane *et al.*, 1938, pp. 52–54).

The mud banks appear to play an important part in the biology and chemistry of the sea-water in these parts, details of which will be referred to in the appropriate sections.

About 200 miles westward of the coast, a chain of coral islands, the Laccadives, is present extending north-south and the portion of the sea between these islands and the mainland is known as the Laccadive Sea. At Calicut itself (Admiralty, 1950) there are a few reefs present which remain submerged. They are situated about 2–3 miles south of the sampling stations of the present investigation.

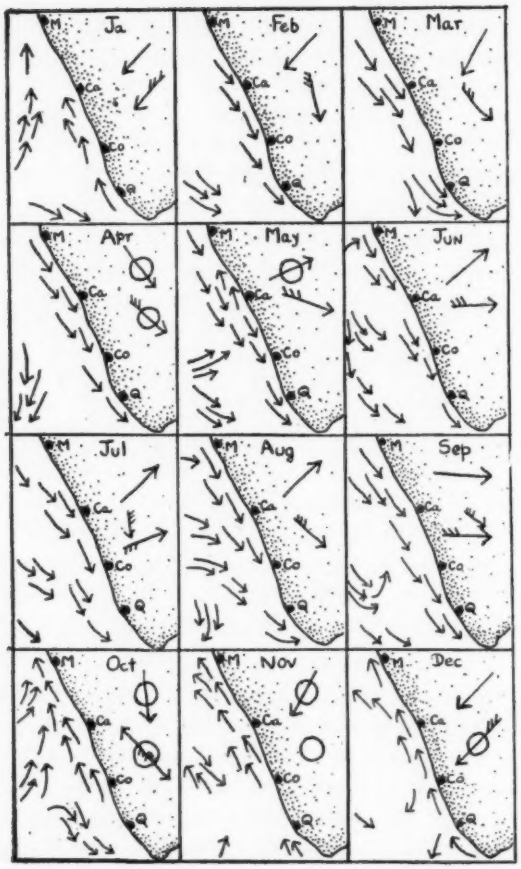
3. *Ocean Currents* (Admiralty, 1950; Sewell, 1929; Möller, 1929) ; (Text-Fig. 2)

In the northern part of the Indian Ocean, the monsoons develop seasonal surface currents in opposite directions, according to the time of the year. The east-going Equatorial Countercurrent, however, though lying mainly south of the Equator extending a few degrees north of the Equator, is not reversed.

During the south-west monsoon, the coastal current in the Arabian Sea and Bay of Bengal sets in a clockwise direction owing to the coastal conformation; and in a counterclockwise direction from November to January, during the height of the north-east monsoon. In the more open parts of the seas, the current takes the direction of a drift current due to the monsoon blowing at the time. This direction is easterly during the south-west monsoon and westerly during the north-east monsoon.

From February to April when the north-east monsoon is weakening, the current circulation of the Arabian Sea and Bay of Bengal has a special character differing from the two main monsoon periods. The south-west monsoon circulation obtains from May to September; October is a month of transition. From November to January, off the west coast of the Indian Peninsula, the predominant direction of the current to about 20° N. is north north-westerly; then north-westerly becoming west north-westerly along the Makkram coast. Off the Arabian coast it is south-westerly.

From February to April, the predominant flow in the open waters is westerly or north-westerly. About the end of January, the counterclockwise



TEXT-FIG. 2. Plan showing currents and prevailing winds. M = Mangalore; Ca = Calicut; Co = Cochin; and Q = Quilon. Winds shown thus ↙ (Arabian Sea) and thus ↙ (West coast). Currents shown thus →. Winds changing thus O. In Nov. O = Winds all round (Adapted from Bristow in Du Cane *et al.*, 1938).

circulation of November to January ceases, and a coastal current in the opposite direction (clockwise) is gradually established; thus, the coastal circulation is reversed while the north-east monsoon is still blowing. The monsoon begins to wane from the beginning of March.

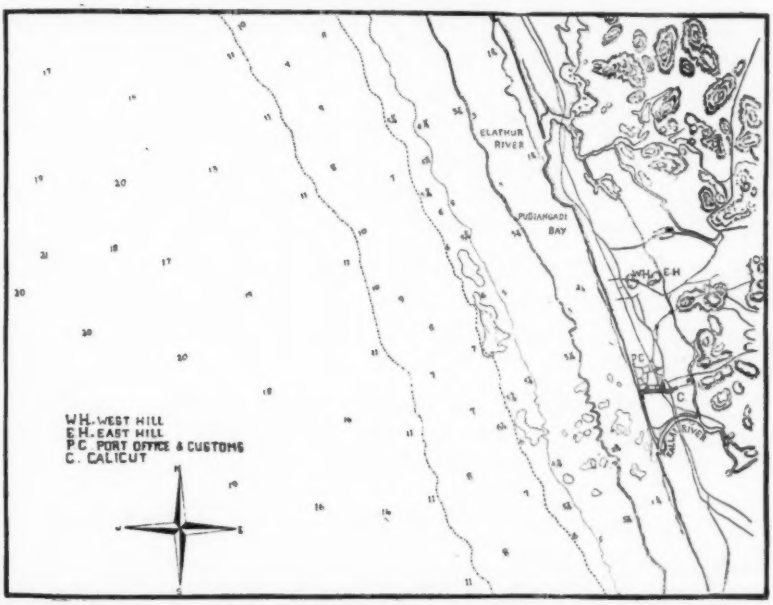
The reversal of the coastal current is simultaneous along all parts of the sea. This reversal, in the Arabian Sea, during the latter part of the north-east monsoon, is attributed to the formation of a gradient current, caused by

temperature differences. By April, before the gradient current diminishes from diminishing temperature difference, the south-west monsoon begins and strengthens the clockwise coastal circulation as a normal current resulting from wind. This resulting coastal current is in continuity with the East African coastal current and this clockwise circulation obtains from May to September. Though there are often variations in the monsoonal drifts, the clockwise coastal circulation during the south-west monsoon is the most constant.

It may be mentioned here that an arm of the Cold Antarctic Flow surfaces in the Arabian Sea (Carpenter, 1887; Sewell, 1925, pp. 47–48) and further, the waters of the Antarctic Intermediate Currents, which rise from great depths towards the surface as they move along the East African coast, also enter the current circulation of the Arabian Sea (Mohamed, 1940, p. 193).

IV. Methods

Collections of plankton were obtained from four fixed stations in the Laccadive Sea off Calicut coast. These stations were situated from 2–12



Text-Fig. 3. Map of Calicut coast showing area of investigation. The numbers represent depth in fathoms (Adapted from Marine Survey of India Map. Figure by courtesy of Shri M. P. Laxman).

miles from the shore and the depth there varied from 4 fathoms (7·5 metres) to 20 fathoms (38 metres); station A at 8 fathoms, B at 4 fathoms, C at 10 fathoms and D at 20 fathoms (Text-Fig. 3). The last could not be worked during the height of the south-west monsoon season owing to inclement weather. It may be mentioned here that most of the commercial fishing along this coast is carried out within this area.

For a relative quantitative assessment of the fluctuation of the plankton, vertical hauls of plankton with a half-metre bolting silk (No. 16) net from a known depth to the surface, was used. This net was similar to the Harvey net (Harvey, 1934 *a*) in construction but with no meter attached. The employment of vertical hauls for quantitative work appears to be reliable (Gardiner, 1931; Russel and Coleman, 1931, 1934) and the values are roughly comparable to the true value of the standing crop (Hart, 1942, p. 277) and the estimate of the standing crop is more accurate than that made with a horizontal or an oblique net (Cushing, 1955, p. 11). Surface and oblique hauls with a $\frac{3}{4}$ metre net made of Organdie cloth have also been studied from the qualitative and taxonomical points of view.

Water samples were also collected from different stations and depths for a detailed and accurate assessment of the quantity of plankton; this also provided a reliable standard for comparison of the magnitude of the standing crop of different geographical regions where similar work had been done.

The quantity of phytoplankton was estimated by enumeration using a Sedgwick-Rafter counting cell and also by the extraction of the plant pigments with acetone according to Harvey's (1934 *a*) method. Other special techniques employed are mentioned in the appropriate sections.

It may be mentioned here that this appears to be the first time that such a quantitative assessment of the plankton, particularly phytoplankton, has been attempted in a tropical environment over a good length of time employing standard methods.

Simultaneously with the study of the plankton, the physical and chemical properties of the sea-water were also under observation. The results of these studies will be reported in a later account.

V. SEASONAL FLUCTUATION IN THE QUANTITY OF PHYTOPLANKTON

1. *Results of Enumeration*

With the signs of the oncoming south-west monsoon, the pre-monsoon thundershowers, the number of phytoplankton elements in the sea begin to increase (Table II). The peak of the bloom, judged by numbers, is attained

TABLE II

Station A. Total number of phytoplankton

Year / Month	1949–50	1950–51	1951–52	1952–53	1953–54	1954–55	Average of 5 years
May ..	18,148,800	34,330,610	65,629,000	16,761,600	263,358,000	44,743,680	79,646,000
June ..	1,809,498,000	97,991,280	1,257,565,200	31,459,000	2,159,592,000	7,705,592,000	1,070,621,000
July ..	1,025,053,000	423,372,320	429,390,000	252,933,800	14,892,665,000	5,346,128,000	3,404,683,000
August ..	1,156,526,000	29,016,800	88,641,400	77,083,400	3,505,344,000	264,768,000	970,722,000
September	250,938,600	157,726,580	148,557,400	103,783,500	5,497,480	35,808,000	133,536,000
October ..	126,394,130	77,918,100	332,686,000	123,984,640	6,053,500	424,704,000	134,361,000
November	18,184,190	50,391,360	11,275,200	38,143,200	5,508,400	..	24,701,000
December	123,381,720	49,248,000	30,198,720	28,022,880	10,046,200	..	48,180,000
January ..	25,573,980	273,047,000	12,530,400	176,208,000	12,619,100	..	100,003,000
February	38,704,240	46,028,950	33,376,800	225,684,000	11,342,400	..	71,027,000
March ..	146,026,560	1,725,600	21,268,100	343,600,000	6,182,400	..	103,799,600
April ..	2,432,490	151,363,600	22,263,000	391,664,000	6,564,000	..	114,858,000

either in June (1949, 1951) or July (1950, 1952, 1953 and 1954) when the monsoon is most active. In some years, 1950 and 1952, the monsoon peak finds expression as a bifid one owing to the drop in the number of phytoplankton elements in August and a rise in September. As the season advances, the number of phytoplankton elements decreases somewhat, particularly the Diatoms, and those of the other elements increase a little; at the same time, there is a fall in the total number of plankton also.

During the period of maxima, the Diatoms constitute 97–99%, the Dinophyceæ 0·5–1·5%, the other phytoplankton elements (Silicoflagellateæ, Coccolithineæ, Cyanophyceæ, etc.) 0·5% and the zooplankton 0·4–0·8% of the total number of organisms in the plankton.* The bulk of the plankton at this time is quite obviously the phytoplankton with the Diatoms dominating the same.

By October–November, the north-east monsoon begins, most of this early period being a transition one. During this season also, the phytoplankton attains one (January 1951; April 1953; and January 1954) or two (December 1949 and March 1950; December 1951 and February 1952) peaks but of a lesser magnitude compared with those during the south-west monsoon season. During the period, at times other than those of peaks of phytoplankton development and generally during the season, the *bulk* of the plankton is contributed by the zooplankton elements. The composition in terms of number, however, is as follows: Diatoms, 92–97%; Dinophycæ, 1–5%; other algæ 0·3–0·8%; and zooplankton 1–1·5%. The elements other than the Diatoms show an increase over the previous season by 1½–6 times, 2–11 times and 2–4 times respectively taking the whole period of investigation under report into consideration.

The phytoplankton content during September to April, 1953–54, was very poor compared with the corresponding periods in the earlier years. This is probably due to the grazing effect on *Fragilaria oceanica* Cleve and other phytoplankton elements by the large shoals of the oil-sardine (*see below*). The fishery of the oil-sardine during that year was one of the best for over a quarter century (Nair and Subrahmanyan, 1955).

2. *Results of Plant Pigment Analyses*

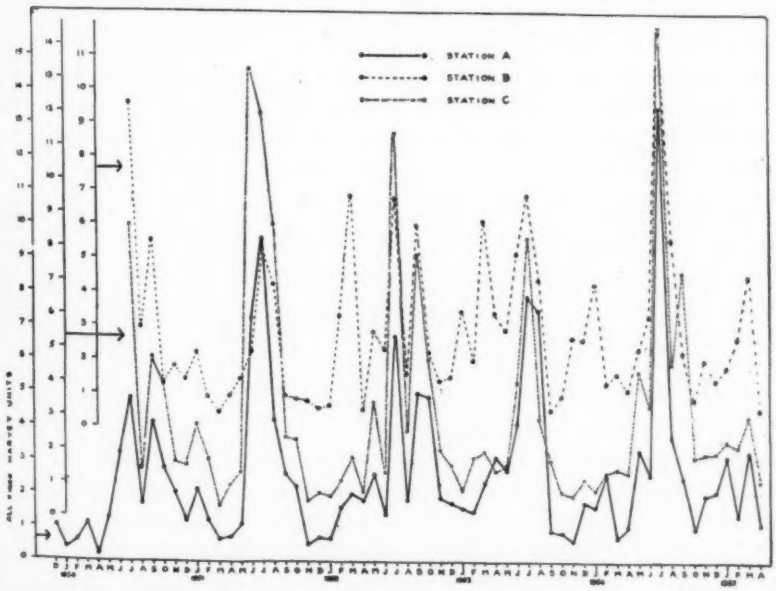
The fluctuation in the quantity of phytoplankton in terms of Plant Pigment Units or Harvey Units follows generally the pattern shown by their

* In the estimation of the number, the total number of cells have been reckoned; in the case of the colonial forms also the number of cells have been determined except in the case of the palmelloid Crysophyceæn alga, which, however, was not of common occurrence.

numbers; a direct proportional relationship could not, however, be established as the size of the cell and the pigment content of the floral constituents of the plankton vary considerably. The results are shown in Text-Fig. 4.

The main peak of development of phytoplankton occurs in July during the south-west monsoon season. In 1950 and 1952, the main peak may be said to be bifid, there being a fall in August and a rise in September, similarly noticed in the estimation in terms of number also. After August or September, the pigment value falls and later in the north-east monsoon period, the value oscillates and peaks of lesser magnitude occur. There is no constancy as to the time of occurrence of this secondary maximum; this may occur any time after the first principal peak between the following January and April. Secondary blooms were noticed in January 1951, March 1952, April 1953, February 1954 and January and March 1955 during the years of investigation.

During this season, the pigment extract, on some occasions, was not of its characteristic green colour and did not match well with the standards



TEXT-FIG. 4. Seasonal fluctuation in the quantity of phytoplankton in terms of Harvey Units (plant pigment units) at Stations A, B and C (Explanation in text).

unlike those during the earlier south-west monsoon season. This is very probably due to the contamination caused by the pigments of zooplankton elements, especially those of Copepods, which form the major portion of the zooplankton bulk during the season.

The trend of fluctuation during the course of several years (Text-Fig. 4) shows a rhythm in the seasonal production of phytoplankton in the water which is very striking when the peaks of production during the south-west monsoon season are considered. The main peaks of the alternate years, 1949, 1951 and 1953, are of a similar type. The bifid peaks in 1950 and 1952 resemble each other more than those of the earlier mentioned years. These resemblances are better brought out when the pigment values are considered than in terms of number. It is clear from the data on hand extending over five years that there is a variation in the intensity of the main peak of production of phytoplankton from year to year. A similar feature is evident in the plankton production between the Yorkshire coast and Dogger Bank (Wimpenny, 1944).

A study of the quantitative fluctuation of the different constituent elements of the plankton (unpublished) has shown that this variation is due to the blooming and dominance of different species of Diatoms during the period concerned, no two years being identical in this regard; further, the factors governing the blooming of the species themselves are internal and concern the life-history of the forms concerned. Some points about this aspect will be dealt with when dealing with the factors influencing phytoplankton production.

The enumeration method in quantitative studies is the most widely employed method and the oldest. The pigment extraction method is a more recent one and while this has been extensively employed in Europe and America, there are only a few similar accounts for the warmer waters of the world. Gilson (1937) appears to be the first to make use of this method in a warmer region; but his results, unfortunately, are not strictly comparable as they are based on samples taken at different stations during a cruise. Ramamurthy (1953 *b*) employed Harvey's method at Madras for the period 1951–52, but as he has not made use of a standard haul for collections (surface horizontal hauls have been used) his results are not comparable with the present investigation. Kow (1953) estimated successfully the phytoplankton of the Singapore Straits for two years (1948 and 1949—almost the same time when the present investigation was on here) in terms of Harvey Units. The results have similarity with those obtained by the writer. The seasonal fluctuation recorded by Kow in terms of numbers and pigment units are

broadly comparable, there being peaks of abundance in April–May, July–August, October–November and sometimes in February. It seems to the writer that attempts at correlating the pigment content with the number of organisms is only of theoretical interest and not of any practical value as the composition of the flora exhibits unforeseeable variations which cannot be handled for relative assessment by two different methods.

Pigment values of known volume of the water sample (5 litres) have also been estimated during this period and these agree fully with the trend of the pigment values obtained based on samples by net collection (Table X).

VI. Quantitative Assessment of the Standing Crop of Phytoplankton and Zooplankton in a Unit Volume of Water Compared with Earlier Observations

1. *Phytoplankton*

An attempt at assessing the magnitude of the standing crop in terms of an internationally accepted standard can now be made to help comparison with the other regions from where such data are available. As the standing crop reflects the magnitude of production, a relative idea of the fertility of the different regions may thus be obtained.

For this purpose, the method adopted was as follows: From the region wherefrom net samples of plankton were collected, some large quantity of water was also collected from the surface, mid-depth and nearer the bottom. Equal known volumes of water from the three samples were filtered through a sieve of bolting silk of the same kind used in the plankton net. The plankton retained by the sieve was estimated by Harvey's method. A volume of the filtrate obtained after the above treatment was filtered through a No. 40 filter-paper and the sediment extracted by acetone to estimate the pigment content. By taking both values into consideration, the phytoplankton content in terms of Harvey Units in a metre cube of water was calculated, assessing the water samples from the three depths as a whole, equal volumes of which had been taken for filtration. Thus water from varying depths and distance from the shore has been analysed for sometime and the data recorded are given in Table X.

The results of the water sample analysis show that during the south-west monsoon season, the phytoplankton dominant period, the fluctuation in the quantity of phytoplankton varies from 11,000–2,48,000 Harvey Units per metre cube of water. During the north-east monsoon season, the range was between 4,000 and 50,000 Harvey Units per M^3 of water. The former

period, obviously, has high phytoplankton content. The monthly average, during the south-west monsoon season, varied from 24,500 Harvey Units per M^3 of water in May to 92,800 Harvey Units per M^3 of water in July; during the north-east monsoon, the monthly average fluctuated between 12,714 Harvey Units per M^3 of water in November to 27,750 Harvey Units for the same quantity of water in January. It is seen that the standing crop is at its highest in July and gradually diminishes reaching the minimum in November, after which it increases somewhat, the quantity oscillating; in January, a small secondary peak may be distinguished; from then on the quantity fluctuates recording higher values gradually in alternate months. The results obtained by the analysis of water samples for plankton fully confirms those obtained by the employment of the net. The high phytoplankton content of the water obtained by the water sample method is striking compared with the lower values for net samples where, obviously, a large number of minute organisms escape through the meshes of the net. However, the net method is less tedious, more rapid and saves considerable time allowing at the same time a rapid relative assessment of the standing crop between stations or regions.

A back calculation taking into account the pigment values obtained by straining a known quantity of water through the silk sieve of the same kind used in the net, and the values obtained by vertical net hauls shows that the quantity of water filtered by the net varies from 24–460 litres (the extremes), the lower value (rare) obtaining during the season of high phytoplankton content and when the elements of the plankton are such as to cause clogging of the net. The average quantity of water strained by the net during a single haul works out to 120 litres of water. So, if the net values are multiplied by, say, $8\frac{1}{3}$, we get approximately the value for one cubic metre of water strained. It may be mentioned that the volume of water passing through Harvey's metred net when hauled vertically from 50 metres to the surface is approximately only 1 metre cube of water (Harvey *et al.*, 1935). This would show that the figure arrived at by calculation for a vertical haul from 15 metres to the surface may generally be accepted for comparisons on a broad basis. Any error present is likely to be one of an underestimation only.

The mean variation (5 years' average) of phytoplankton content of net samples in Harvey Units is between 1,412 in November to 8,002 in July at Station A; 1,202 in October to 8,064 in July at Station B; and, 1,092 in April to 10,970 in July at Station C (Text-Fig. 4). In Table III, the calculated quantity of phytoplankton in terms of Harvey Units is shown for the net samples from Station A (the values from other stations are comparable).

TABLE III

Number of different groups of plankton organisms in 1 litre of sea-water and total quantity of phytoplankton in Harvey Units (based on the data from 5 years' average). Calculated values

Station A

Month	Diatomaceæ in 1 litre	Dinophyceæ in 1 litre	Miscellaneous Algae in 1 litre	Zooplankton in 1 litre	Phytoplankton in Harvey Units	
					In 1 M^3	Under 1 metre-sq. of sea surface
May ..	655,520	7,025	1,164	3,358	18,366	275,490
June ..	8,896,300	25,530	2,026	9,525	30,700	460,500
July ..	28,349,590	21,550	1,229	11,308	66,684	1,000,260
August ..	8,074,600	14,275	471	11,608	31,516	472,740
September ..	1,106,450	5,640	709	3,050	25,166	377,490
October ..	1,105,690	13,825	145	3,442	19,516	292,740
November ..	193,910	11,517	409	3,825	11,766	176,490
December ..	391,100	9,683	703	3,583	12,600	189,000
January ..	827,060	5,692	600	6,175	10,634	159,510
February ..	587,600	3,392	894	3,183	12,634	189,510
March ..	856,730	6,625	1,641	4,317	11,416	171,240
April ..	945,050	10,000	2,103	5,083	11,416	171,240

It may be seen that the values are of a high order (the calculations are based on the overall five years' average figures). The lowest value recorded here is comparable to those recorded from some of the highly productive regions of the world as will be shown later. The pigment content ranges from over 10,000 to over 65,000 Harvey Units per metre cube of water. In the case of the water sample data, the monthly average varies from 12,700–92,800 Harvey Units per metre cube of water. The value by both methods are almost comparable. The higher values obtained in the analyses of water samples are due to the more efficient method of filtration employed. It becomes quite evident that a large number of organisms escape through the meshes of the net and is not estimated in the ordinary hauls.

In connexion with the assessment of the standing crop made above, some of the data obtained in investigations in other parts of the world may be compared as these would indicate the relative fertility of the waters concerned. In Tables IV and V, the data from several geographical areas in terms of plant pigment units, and in Tables VI, VII, VIII and IX, in terms of numbers of organisms, total as well as for constituent classes, are presented along with those recorded from the present area on the west coast of India. It may be seen from the tables that the values recorded here are of a very high order, comparable to those from some of the highly productive areas of the world.

It may be mentioned here that the season of the year when phytoplankton bloom occurs in the different regions in relation to the calendar months varies, *i.e.*, during summer in the northern hemisphere, it is winter in the southern hemisphere, and this fact needs to be borne in mind in the study of the data presented in the tables. In the tropics, however, such seasonal changes as occurring in the temperate and Arctic regions are not so well pronounced. The period of the main bloom of phytoplankton in the temperate and cold regions is in the spring and may be followed by a second bloom late in the summer or autumn. In the present area, in the tropics, the main bloom occurs in the summer, in June–July, the south-west monsoon season; and, a second bloom may occur during the north-east monsoon season, this falling either in "winter' or "spring" months for the northern hemisphere. In general, here, only a dry and wet season are distinguishable.

2. *Zooplankton*

In connexion with the assessment of the magnitude of the standing crop, it may be interesting to mention here for comparison a few observations on the zooplankton also, from other areas. Unfortunately, there are not many accounts that are directly comparable as either the methods employed

are different, or the values expressed are in terms of a different standard or the zooplankton is treated as groups instead of as a whole.

As in the case of phytoplankton, considerable work has been done in the temperate and cold regions on the qualitative and quantitative aspects of the zooplankton. For the tropical and sub-tropical regions, there are only some papers (Graham, 1941 *a*; Krämer, 1906; Jespersen, 1935; Matsuya, 1937; Motoda, 1940; Haneda, 1942; Tokioka, 1942 *a*) and reports of some of the expeditions (*Challenger*, *Albatross*, *Meteor* and so on); the results of most are not based on continuous work in any one region but on samples taken at intervals of time and distance, latitude- and longitude-war; most contain only qualitative data.

In Table XI, the data from several accounts are summarised for comparison with results obtained here. It may be seen from the table that the values recorded at Calicut are of a very high order indicating a high productivity as in the instance of phytoplankton. Again, that the zooplankton content of the water here could be fairly high may also be seen from the comparison with the quantity (number) of zooplankton recorded here with some accounts relating to areas where continuous work has been done. Table XII shows the data as number of organisms in 100 litres of water from the English Channel, Kiel Bay (Harvey *et al.*, 1935, p. 424) and Calicut, the present area (*cf.* also Table III). It may be seen that the standing crop of zooplankton at Calicut is very high as was the case with the phytoplankton. The sea off the west coast of India, may, therefore, be regarded as highly fertile.

VII. Magnitude of the Standing Crop in Terms of Carbon per Metre Square of Sea Surface

The ratio among Phosphate-P, Nitrate-N and Silicate-Si is 1 : 15 : 20 on the west coast of India as obtaining in the temperate regions (Subrahmanyan, Part II). From the constancy of these elements (N, P and Si) and the ratio at which they are present in the water and plankton, Fleming (1940) has worked out equivalents from which one could, based on one datum, evaluate the same in terms of some other factor. Thus one pigment unit of plankton represents $3{\cdot}3 \times 10^{-3}$ milligrams of carbon; so, the standing crop of phytoplankton here can also be evaluated in terms of carbon. Table X shows carbon values calculated in this manner from the pigment content of the water samples, in order, the values may be compared with the data from other areas. As may be seen, the standing crop in terms of carbon varies from 0·0627 gram to 12·280 grams over a metre square area of sea

TABLE

Comparison of the magnitude

[Figures in thousand ; figures within brackets

Area	Year	January	February	March	April
1 South-west coast of India Net plankton at 15 metres depth off Calicut shore (Calculated values)	1949–55 (Average of 5 years observations)	159 (20)	189 (20)	171 (20)	171 (20)
2 do. water samples off area at 15 metre depth	1955–56	413 (8)	253 (8)	365 (9)	297 (9)
3 Gulf of Maine ..	1939	..	..	..	..
	1940	85 (1)	..	310 (1)	620 (1)
	1941	..	820 (1)	820 (1)	690 (1)
4 Georges Bank ..	1939	..	..	..	..
	1940	110 (8)	..	1040 (13)	2250 (15)
	1941	..	..	350 (29)	1390 (31)
5 Coastal water ..	1939	..	..	..	..
6 Cape Cod-Montauk Point ..	1947	..	..	460 (3)	..
7 Slope water ..	1939	..	..	..	..
	1940	250 (2)	..	470 (1)	3800 (1)
	1941	..	..	890 (1)	3540 (2)
	1947	..	..	370 (1)	..
8 Sargasso Sea :					
North-west Bermuda ..	1939	..	..	..	..
	1947	..	..	..	..
Bermuda to Azores ..	1947	..	..	..	..
	1948	..	..	..	..
9 Gulf Stream System :					
Florida Straits ..	1937	..	..	..	..
	1939	..	..	..	..
Carolinas ..	1939	..	..	..	..
Off Montauk Point ..	1939	..	..	..	..

IV

of the standing crop

indicate number of observations]

May	June	July	August	September	October	November	December
275 (20)	460 (15)	1000 (12)	472 (20)	377 (20)	292 (20)	176 (20)	189 (20)
367 (6)	900 (4)	1392 (6)	1373 (10)	835 (8)	589 (8)	191 (7)	222 (10)
..	..	..	..	560 (2)	..	..	..
..	..	..	..	..	..	..	..
1230 (2)	490 (2)	..	..	..	..	..	..
..	..	..	..	560 (22)	..	..	..
860 (20)	500 (28)	..	..	..	..	..	..
1280 (29)	330 (29)	..	..	..	..	..	..
90 (2)	..	..	..	..	..	..	..
..	..	..	..	..	..	..	200 (3)
220 (2)	..	..	..	380 (2)	..	..	..
1180 (1)	250 (2)	..	..	..	..	..	..
270 (2)	340 (1)	..	..	..	..	..	..
..	..	..	..	..	..	..	300 (1)
310 (5)	..	..	..	..	..	..	..
..	..	..	..	..	..	..	410 (2)
..	..	..	50 (5)	..	..	..	420 (7)
..	100 (3)	..	..	..	..	..	..
..	..	..	100 (1)	..	..	..	..
480 (5)	..	..	..	..	..	..	..
370 (2)	..	..	..	..	..	..	..
300 (1)	..	..	..	..	..	..	..

TABLE V

Comparison of the magnitude of the standing crop

Region or Area	English Channel* 0–15 metres ?	*Antarctic waters (Hart 1942*)					Georges Bank (Riley, 1941)
		Northern	Intermediate	Southern	South Georgia	Scotia Sea	
Period ..	1st March to 3rd July 1934	27th September to 1st February, 1938–39	27th November to 8th March	20th January to 4th March	13th October to 27th January	6th October to 15th February	March to June
Number of observations	16	61	87	42	72	55	24
Mean Units per metre-cube of sea-water	1,000	1,210	1,100	1,150	11,690	2,390	11,670 (Average of observations at different depths)
Highest individual observation	3,850	7,540	9,420	12,050	60,040	21,040	65,000

Region or area	North central part of Long Island Sound				Calicut 0–15 metres		Singapore (Kow, 1953)	Block Island sound (Riley, 1952)
	20 metres or less (Riley, 1952)				Net sample	Water sample		
Period ..	September 7th 1938 to August 18th, 1939	July 8th to November 13th, 1940	June 16th to September 22nd, 1941	March 17th to July 26th, 1950	June to October (5 years data-average)	July to October, 1955	1948 and 1949	January and December
Number of observations	65	54	51	9	About 100 (20 in all for each month	32	..	..
Mean Units per metre-cube of sea-water	*ca*55,600	45,200	45,600	31,500	32,000†	66,900	1500 and 2500 respectively	11,200
Highest individual observation	194,000	125,000	79,000	75,000	147,300†	248,000	..	26,000

* Data from Hart, 1942 † Calculated values.

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* Data from Hart, 1942 † Calculated values.

TABLE VI

Total number of phytoplankton in a litre of sea-water

Month	Aomori Bay (Kokubo, 1932)		Woods Hole (Lillick, 1937)	Calicut, net samples Average of 5 years, calculated	Calicut, water sample centrifuged
	1929	1930			
January ..	6,460	780	182,000	833,460	..
February ..	19,430	560	19,200	591,900	..
March ..	15,280	40,300	1,200	865,000	..
April ..	5,960	46,470	20,900	957,200	..
May ..	6,230	39,230	12,000	663,700	..
June ..	10,940	14,320	32,400	8,921,800	..
July ..	21,780	13,400	111,800 10,400	28,372,400	18,081,600
August ..	4,640	87,600	143,100 12,400	8,089,350	4,120,700
September ..	64,270	10,800	12,800	1,112,800	1,921,500
October ..	5,140	85,800	305,000	1,119,700	9,604,800
November ..	2,280	55,780	60,300	205,800	..
December ..	12,290	29,300	13,000	401,500	..

TABLE VII

Total number of Diatom cells in one litre of sea-water

Month	Calicut Average of 1949–54	Low Isles* Series 1 1928–29	Plymouth* 1915–16	Loch Striven* 1926	Kiel Bay* 1905–06	La Jolla* 1922	Calicut 1954 Water sample centrifuged
January ..	827,060	4,750	2,190	7,000	4,100	5,920	..
February ..	587,600	2,070	1,620	34,000	4,800	19,170	..
March ..	856,730	5,180	2,070	1,356,000	48,000	13,310	..
April ..	945,050	5,440	38,560	3,792,200	850,000	312,510	..
May ..	655,520	5,470	30,420	1,686,900	2,100,000	11,770	..
June ..	8,896,300	6,390	9,120	585,500	2,480,000	23,220	..
July ..	28,349,590	5,780	21,390	620,900	37,000	2,650	18,071,000
August ..	8,074,600	17,810	31,780	2,774,900	130,000 (44,000)	590	4,085,600
September ..	1,106,450	5,310	17,110 (2,990)	693,650	1,400,000	2,880	1,918,000
October ..	1,105,690	2,990	17,410	179,000	440,000	2,530	9,572,850
November ..	193,910	5,040	9,500	196,000	15,000	85,290	..
December ..	391,100	3,280	1,500	186,000	8,500	110	..

* Data from Marshall, 1933.

TABLE VIII

* Data from Marshall, 1933.

TABLE VIII

Total number of phytoplankton beneath 0·1 metre-square of the sea

Month	Gulf of Maine (Bigelow, Lillick and Sears, 1940)				Calicut (Net samples)		Calicut (Water sample) (Single observations)
	Mean	Maximum	Minimum	Mean (based on 5 years data)	Maximum	Minimum	
January ..	6,026,000	12,073,000	1,672,000	1,250,190,000	7,877,000,000	10,665,000	..
February ..	..	..	..	887,850,000	5,356,000,000	1,413,000	..
March ..	29,487,000	117,425,000	550,000	1,297,500,000	18,420,000,000	930,000	..
April* ..	1,759,767,000	13,965,600,000	80 000	,435,800,000	13,044,000,000	870,000	..
May† ..	55,347,000	142,100,000	50,000	995,550,000	12,837,600,000	346,500	..
June ..	6,650,000	27,310,000	1,120,000	13,382,700,000	267,183,000 000	12,388,000	..
July‡ ..	21,685,000	198,080,000	800,000	42,553,600,000	667,248,250,000	230,400,000	27,122,400,000
August ..	..	..	..	12,134,250,000	124,236,000,000	32,875,000	6,181,050,000
September ..	37,660,000	283,012,000	2,013,000	1 669,200,000	6,096,500,000	6,600,000	2,882,250,000
October ..	7,856,000	18,161,000	649,000	1,679,550,000	19,838,400,000	12,240,000	14,407,200 000
November ..	..	..	..	308,700,000	1,868,832,000	481,500	..
December ..	1,852,000	3,230,300	80,000	602,250,000	3,257,970,000	2,310,000	..

*, †, ‡: Gulf of Marine data for April-May, May-June and June-July.

Data From Lohman, 1920.* (Mean values for water column)

Month	Atlantic 40–50° N. and 10–30° W.	Atlantic 40–30° N.	Atlantic 30–20° N.	Atlantic 20–10° N.	Atlantic 10–0° N.
May ..	240,000,000	80,000,000	24,000,000	20,000,000	24,000,000
June ..	240,000,000	80,000,000	24,000,000	20,000,000	24,000,000
? ..	..	..	..	75,000,000†	46,000,000†

* Data as given by Riley *et al.* (1949). † Hentschel's (1932) data as given in Riley *et al.* (1949).

TABLE IX

Total number of Dinophyceæ cells in one litre of sea water

Month	Calicut Average of 1949–54	Low Isles* Series 1 1928–29	Plymouth* 1915–16	Loch Striven* 1926	Kiel Bay* 1905–06	La Jolla* 1922	Calicut 1954 Water sample centrifuged
January ..	5,692	960	3	400	7,000	460	..
February ..	3,392	780	2	300	4,800	360	..
March ..	6,625	450	20	1,800	16,000	820	..
April ..	10,000	350	30	6,940	18,000	4,770	..
May ..	7,025	680	120	6,250	31,000	5,140	..
June ..	23,530	860	1,020	8,240	187,500	2,230	..
July ..	21,550	1,060	120	3,960	382,000	600	10,600
August ..	14,275	1,470	730	10,000	146,000 (105,000)	470	35,080
September ..	5,640	1,020	120 (640)	6,400	19,000	790	3,550
October ..	13,840	970	80	7,700	6,600	1,040	31,990
November ..	11,518	860	20	3,430	14,000	1,130	..
December ..	9,683	1,110	8	1,600	7,400	600	..

* Data from Marshall, 1933.

TABLE X

Standing crop of phytoplankton as estimated from water samples by Harvey method and in terms of carbon per square metre of sea surface

Date	Sampling station-depth in metres	Number of Harvey Units per metre-cube of water	Number of Harvey Units under one metre-square of sea	Number of grams carbon under one metre-square of sea	Monthly average H.U./M³
4- 7-1955	15	160,000	2,400,000	7·920	
7- 7-1955	19	20,000	380,000	1·254	
7- 7-1955	0	(4,000)	76,000	0·251	
8- 7-1955	11·25	15,000	168,750	0·557	
11- 7-1955	19	20,000	380,000	1·254	
22- 7-1955	3·75 (Quilandy)	80,000	300,000	0·990	
28- 7-1955	15	248,000	3,720,000	12·280	92,800
1- 8-1955	15	110,000	1,650,000	5·445	
4- 8-1955	19	28,000	532,000	1·756	
8- 8-1955	19	100,000	1,900,000	9·017	
10- 8-1955	22·5	47,000	1,057,500	3·489	
11- 8-1955	33·75	24,000	810,000	2·673	
16- 8-1955	19	76,000	1,444,000	4·765	
17- 8-1955	11·25	216,000	2,430,000	8·166	
18- 8-1955	33·75	32,000	1,080,000	3·565	
22- 8-1955	19	172,000	3,268,000	10·780	
25- 8-1955	33·75	110,000	3,712,000	12·260	91,500
1- 9-1955	28	80,000	2,240,000	7·392	
5- 9-1955	19	55,000	1,045,000	3·449	

• Data from Marshall, 1933.

TABLE X (*Contd.*)

Date	Sampling station-depth in metres	Number of Harvey Units per metre-cube of water	Number of Harvey Units under one metre-square of sea	Number of grams carbon under one metre-square of sea	Monthly average H.U./M^3
8– 9–1955	30	90,000	2,700,000	8·910	
12– 9–1955	19	80,000	1,520,000	5·017	
15– 9–1955	33·75	43,000	1,451,000	4·788	
20– 9–1955	19	68,000	1,292,000	4·264	
22– 9–1955	33·75	13,500	455,500	1·503	
26– 9–1955	19	16,000	304,000	1·003	55,688
3–10–1955	19	23,000	437,000	1·442	
6–10–1955	33·75	40,000	1,350,000	4·454	
10–10–1955	19	65,000	1,235,000	4·076	
13–10–1955	33·75	46,000	1,552,000	4·069	
17–10–1955	19	34,000	646,000	2·132	
20–10–1955	22·5	41,000	922,600	3·832	
27–10–1955	19	21,000	399,000	1·317	
31–10–1955	19	44,000	836,000	2·759	39,250
3–11–1955	32	7,000	224,000	0·739	
7–11–1955	19	27,000	513,000	1·693	
10–11–1955	32	13,000	416,000	1·373	
17–11–1955	19	10,000	190,000	0·627	
21–11–1955	19	4,000	76,000	0·0627	
24–11–1955	34	16,000	544,000	2·061	
28–11–1955	19	12,000	228,000	0·7525	12,714

TABLE X *(Contd.)*

Date	Sampling station-depth in metres	Number of Harvey Units per metre-cube of water	Number of Harvey Units under one metre-square of sea	Number of grams carbon under one metre-square of sea	Monthly average H.U./M^3
1-12-1955	34	4,000	136,000	0·4488	
5-12-1955	19	14,000	266,000	0·8778	
8-12-1955	38	24,000	912,000	3·005	
12-12-1955	19	16,000	304,000	1·003	
13-12-1955	11	18,000	198,000	0·6534	
15-12-1955	38	10,000	380,000	1·254	
19-12-1955	19	8,000	152,000	0·5017	
22-12-1955	38	13,000	494,000	1·630	
26-12-1955	19	23,000	437,000	1·442	
29-12-1955	38	18,000	684,000	2·257	14,800
2- 1-1956	19	45,000	855,000	2·821	
5- 1-1956	34	11,400	387,600	1·279	
9- 1-1956	19	36,000	684,000	2·257	
12- 1-1956	38	4,000	72,000	0·5017	
16- 1-1956	19	18,000	342,000	1·129	
19- 1-1956	38	8,000	304,000	1·0034	
23- 1-1956	19	48,000	912,000	3·009	
30- 1-1956	19	50.000	950,000	3·135	27,550
2- 2-1956	32	3,000	96,000	0·3135	
6- 2-1956	19	29,000	551,000	1·8190	
9- 2-1956	34	11,000	374,000	1·234	
13- 2-1956	19	18,000	342,000	1·219	
16- 2-1956	34	18,000	612,000	2·019	
20- 2-1956	19	25,000	475,000	1·568	
23- 2-1956	38	4,000	72,000	0·5017	
27- 2-1956	19	27,000	513,000	1·693	16,875
1- 3-1956	34	16,500	561,000	1·851	

TABLE X (*Contd.*)

Date	Sampling station-depth in metres	Number of Harvey Units per metre-cube of water	Number of Harvey Units under one metre-square of sea	Number of grams carbon under one metre-square of sea	Monthly average H.U./M³
5– 3–1956	19	47,000	893,000	2·947	
8– 3–1956	38	28,000	1,064,000	3·512	
12– 3–1956	19	38,000	722,000	2·383	
15– 3–1956	34	32,500	1,105,000	3·647	
19– 3–1956	19	27,000	513,000	1·693	
22– 3–1956	34	14,000	476,000	1·570	
26– 3–1956	19	13,000	247,000	0·8151	
29– 3–1956	38	4,000	132,000	0·5017	24,556
2– 4–1956	19	13,500	256,500	0·8465	
5– 4–1956	32	32,000	1,024,000	3·378	
9– 4–1956	19	37,000	703,000	2·320	
12– 4–1956	32	20,000	640,000	2·112	
16– 4–1956	19	10,000	190,000	0·627	
19– 4–1956	34	18,000	612,000	2·019	
23– 4–1956	19	19,000	361,000	1–191	
26– 4–1956	30	13,000	390,000	1·286	
30– 4–1956	19	16,000	304,000	1·003	19,833
3– 5–1956	30	11,000	330,000	1·089	
7– 5–1956	19	23,000	437,000	1·442	
10– 5–1956	34	11,000	374,000	1·234	
14– 5–1956	19	19,000	361,000	1·191	
29– 5–1956	15	36,000	540,000	1·782	
31– 5–1956	6	47,000	282,000	0·9307	24,500
4– 6–1956	11	17,000	187,000	0·6175	
11– 6–1956	11	63,000	693,000	2·287	
14– 6–1956	11	102,000	1,122,000	3·702	
18– 6–1956	15	58,000	870,000	2·871	60,000

TABLE XI

Comparison of plankton abundance in various areas of the world

Area	Plankton values Number/volume in c.c.	Mesh apperture of net used	Reference	Remarks
*PACIFIC AREA—				
Samoa to the Marshalls *via* the Equator	0·4 to 0·95 c.c./m³	0·33 mm.	Krämer (1906)	
Java Sea ..	Avg. = 0·83 c.c./m³	0·33 mm.	Delsman (1939)	
Open Sea Palau Is. ..	110 to 530 no./m³	0·33 mm.	Motoda (1940)	
Bikini, just outside reef ..	26·23 to 62·20 no./m³	0·37 mm.	Johnson (1949)	
West coast, U.S. (offshore stations)	0·016 to 0·129 c.c./m³ Avg. = 0·057 c.c./m³	0·65 mm.	California Prog. Rept. (1950)	
Central Pacific ..	5 to 109 no./m³, Avg. = 35 ; 0·002 to 0·102 c.c./m³ Avg. = 0·027 c.c./m³	0·65 mm.	This report (King and Demond, 1953)	
*ATLANTIC AREA—				
Baltic Sea ..	1·9 to 11·0 c.c./m³	0·33 mm.	Krämer (1906)	
Gulf of Maine (10 to 100 metres depth)	0·12 to 4·30 c.c./m³	1·25 mm., front ; 0·8 mm., middle and rear	Bigelow (1926)	
North Atlantic (Coastal water)	Avg. = 0·5 to 0·8 c.c./m³	29·38 meshes/in., front ; 48·54 meshes/in. rear	Bigelow and Sears (1939)	
Florida Strait ..	0·02 c.c./m³	0·158 mm.	Riley (1939)	
Gulf Stream, off Florida ..	0·05 c.c./m³			
Gulf Stream, off Georgia ..	0·07 c.c./m³			
North Atlantic continental slope	4·3 c.c./m³			
North Atlantic, coastal ..	8·1 c.c./m³			
North Atlantic, coastal ..	Avg. = 0·54 c.c./m³ Max. = 15·5 c.c./m³	10 strands/cm.	Clarke (1940)	
North Atlantic, offshore ..	Avg. = 0·40 c.c./m³ Max. = 3·50 c.c./m³			
Sargasso Sea ..	Avg. = 0·045 g./m³	0·158 mm.	Riley and Gorgy (1948)	
Gulf Stream ..	1 sta. = 0·137 g./m³			
Slope water ..	2 sta. = 0·14 and 1·6 g./m³			
†OTHER DATA—				
Near Equator ..	0·5 to 5 c.c./?	?	Tokioka (1942 *a*)	Vertical haul from 50 to 0 m.
30° N. latitude ..	0·25 to 1·75 c.c./?	?	Haneda (1942)	
Hawaiian waters ..	20·0 to 40·0 c.c.	0·65 mm.	King and Hida (1954)	Oblique tow from 200 to 0 m.
Block Island Sound ..	Less than 2 c.c./m³	No. 10 net	Deevy (1952 *b*)	
English Channel ..	0·1 to 4 c.c./m³	?	Harvey *et al.* (1935)	
New South Wales coast ..	Avg. 7·6 c.c.) in Var. 1–28 c.c.) 1943 Avg. 11·7 c.c.) in Var. 2–41 c.c.) 1944	?	Sheard (1949)	
South-east coast of India	Up to 40 c.c. ; avg. much less	Organdy net, *ca.* 36 str./cm.	Prasad (1954 *a*, 1956)	Oblique hauls *ca.* 10 to 0 m.
West coast of India Calicut	11·75 to 32·5 c.c.	do.	George (1953 *a*)	Oblique hauls *ca.* 15 to 0 m.
do.	20 c.c./m³ max.	No. 16 net	Present account	Vertical hauls from 15 to 0 m.

* From King and Demond (1953).

† By writer.

TABLE XII

Zooplankton in 100 *litres of water* (*comparison with other data*)

Months	Calicut, net samples; 5 years average calculated for 100 litres	Calicut, water samples centrifuged material	Plymouth Station L_4, 1934 Harvey *et al.*, 1935	Kiel Bay; Lohman, 1908	
January ..	444,600	..	239	3,700	1906
February ..	229,200	..	545	4,700	
March ..	310,800	..	2,024	6,000	
April ..	366,000	..	2,518	5,800	
May ..	241,800	..	3,365	28,000	
June ..	685,800	..	2,440	7,400	
July ..	814,200	3,730,000	2,852	37,000	
August ..	835,800	4,798,600	4,943	7,600 19,000	1905
September ..	219,600	2,220,000	4,818	24,000	
October ..	247,800	2,308,000	1,839	24,000	
November ..	275,400	..	1,338	13,000	
December ..	258,000	..	736	5,700	

surface, the depth of the sampling stations not exceeding 40 metres. Highest values occur during the south-west monsoon season.

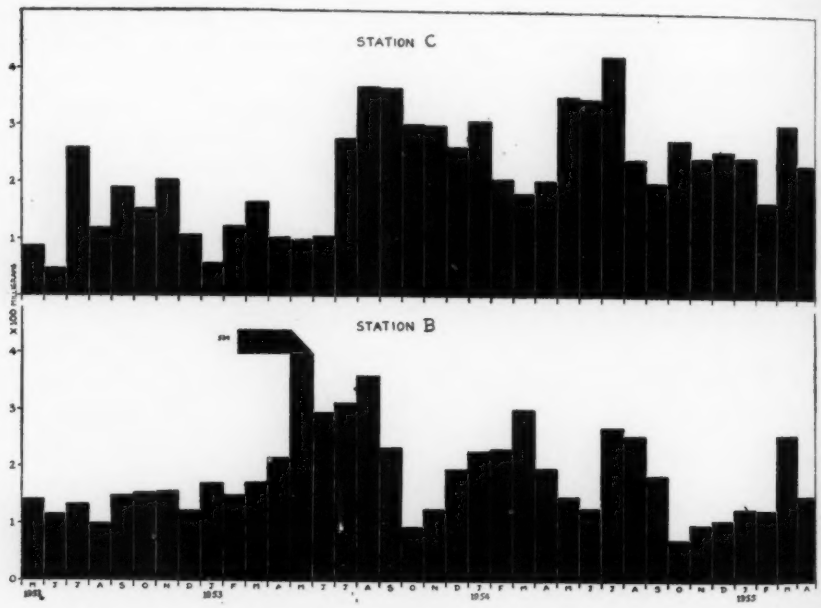
It may be mentioned in this connexion, that a very rich catch of plankton in the English Channel (Harvey, 1945, p. 143) showed 1·5 grams of carbon beneath one metre square of sea surface. In the Gulf of Maine, during the richest period, one metre-square of sea surface contained 2 grams of carbon (Riley, 1941). In the Antarctic (which is one of the most fertile seas of the world) 7·2 grams of carbon for a similar water column has been calculated (Hart, 1942). The richest catch on the west coast of India for a water column of 15 metres depth represented 12·28 grams of carbon per metre-square. It may be reliably stated that the west coast of India is one of the highly productive regions of the world in general and the tropics in particular. This is borne out by all aspects of study—enumeration of plankton content, estimation of plant pigments and of carbon content. Further, it is seen that throughout the year there is a high standing crop, the intensity of which shows clear seasonal fluctuation.

VIII. Seasonal Fluctuation in the Quantity of Total Plankton—Dry Weight

The dry weight was estimated as follows: the whole sample of plankton (vertical haul) was filtered through a No. 40 Whatman filter-paper, dried at 60° C. to constant weight. The method employed for collection and for drying, etc., are almost similar to that employed by Graham (1941 *a*). In this method both phyto- and zoo-plankton are included.

In Text-Fig. 5 is shown the fluctuation of the total plankton content of the catch in terms of dry weight, on the basis of monthly average for a period of three years, at Stations B and C. It may be seen that only on a very few occasions has the quantity per haul fallen below 100 milligrams. At Station B, the monthly overall average for the three years was 148, 259 and 160 milligrams and at Station C, 135, 239 and 275 milligrams respectively for the three years 1952–53, 1953–54 and 1954–55. Such sharp oscillations seen in terms of number and of Harvey Units is absent in this instance and there is also not much difference in the dry weight of plankton during the two main seasons, though the *bulk* of the plankton is phytoplankton during the south-west monsoon period and zooplankton during the north-east monsoon season. Two interesting points stand out here, namely, that in those years when the plankton bulk is poor during the south-west monsoon season, there is a fall during the north-east monsoon season also; secondly, the ratio of the quantity of plankton in terms of dry weight, that of the south-west

monsoon season to that of the north-east monsoon season (totals of May to October and November to April respectively) shows a striking relationship, the values being 1 : 1·2, 1 : 0·7, 1 : 0·8 at Station B and 1 : 0·9, 1 : 1, 1 : 0·8 at Station C for the years 1952–53, 1953–54 and 1954–55 respectively. The overall average of the three years at Stations B and C is the same, *viz.*, 1 : 0·9. Perhaps the slight difference noticed might represent unsampled forms of life in the sea. This relationship is probably an indication that the water column at a particular period (six-month season) is able only to support a more or less fixed amount of life, *biomass* (whether floral or faunal matter) which is somewhat analogous to the equilibrium of some of the constituent elements in the sea-water, *e.g.*, Nitrogen, Silicon and Phosphorus (Sverdrup *et al.*, 1942; Subrahmanyan, Part II). This relationhsip is worth investigating at other centres also.



TEXT-FIG. 5. Seasonal fluctuation in the quantity of plankton in terms of dry weight at Stations B and C (Explanation in text).

Graham (1941 *a*) studied the plankton in the Pacific between 40° N. and 20° S. latitude during a cruise on the *Carniegie*. He showed that there was greater production of plankton in the tropics than in the more northern latitudes. Apart from the fact that the production on the west coast of India is even higher than at the Pacific stations, the striking similarity of the results

obtained from these two investigations is worth noting; however, there is one difference in that the main constituents of Graham's collections appear to be zooplankton, whereas here the collections are of a mixed type. Nevertheless, in view of the close approximation in the ratio of the dry weights between the predominantly phytoplankton period and the later zooplankton period, the present results are definitely comparable with those from the Pacific.

Riley and Gorgy (1948) recorded an average plankton content of 45 milligrams per M^3 of water in the Sargasso Sea, 137 milligrams per M^3 in the Gulf Stream and 140 and 1,600 milligrams at two stations respectively on slope water. All vertical hauls have an average monthly value exceeding 1,000 milligrams per metre cube of water at both Stations, B and C, during all the years of the present investigation. This indicates a very high standing crop for the west coast of India and thus a high fertility for the area.

In a critical review of the utility of the pigment values in quantitative determination of phytoplankton, Margalef (1954) states that the quantity of pigment in the cells is influenced by various factors and, therefore, he believes, that the pigment value alone is not a sound index whether of the standing crop or of productivity, but that the same together with dry weight estimations is able to show most interesting features of the dynamics of production. Estimations of plankton by dry weight in the present investigation have similarly revealed some interesting facts about production of matter in the sea not brought out either by the pigment extraction method or enumeration method.

IX. Horizontal Distribution of Phytoplankton

As already mentioned, samples of plankton from four stations A, B, C and D, varying in distance from the shore, have been studied; for the first three, data are available for over five years; but, for D the data is considerably interrupted as this station could not be worked during the south-west monsoon season owing to inclement weather. Only pigment values have been taken into consideration. The enumeration data are complete only for Station A; as the results of two years' enumeration for B and C showed more or less the same trend in the fluctuation as at A, this method was discontinued.

Text-Figure 4 shows the fluctuation in the quantity of the standing crop of phytoplankton at these stations. It may be seen that, in general, the trend of fluctuation is the same at all stations, that at A and C, stations farther out, being closely identical. At Station B also the trend in the fluctuation of the quantity of phytoplankton is similar except for some minor differences.

At Station B, in 1951–52, the north-east monsoon bloom in March was of a higher order than the south-west monsoon bloom in July. In 1952–53, the north-east monsoon peak was bifid, there being a small peak of production in January and an intense one in March. It may also be noticed that closer the station is to the shore, greater the frequency in the number of oscillations in the quantity of the standing crop. This is probably due to the inshore environment where wind plays a rôle in the mixing up of the water layers more thoroughly than at stations farther off-shore and the consequent increase of certain nutrients from the bottom mud enabling an increased multiplication of the floral elements. A similar feature brought about by wind action has been noticed by Marshall (1933) in the Great Barrier Reef region.

Further, certain differences in the intensity of the bloom of phytoplankton at these stations (as far as it could be judged though there was no means by which the quantity of water strained through the net could be measured directly) were met with frequently. During the south-west monsoon season, at the beginning, the bloom of phytoplankton is of a high order at stations farther away from the shore than at places nearer; later, when the weather conditions become stabilised, the bloom over the whole area is more or less of the same intensity, and the flora also is similar. The differences early in the season may be due to the very low salinity of the shore water. Too low a salinity appears to be injurious to the floral elements.

While there appears to be some uniformity in the composition of the flora during the south-west monsoon season, often striking differences are noticeable during the north-east monsoon in the constituent elements from the different stations. These differences may be due to the fact that currents are unsettled during the early part of the season (October to December) leading to patchy distribution of the plankton organisms. Later in the season, when conditions become more stable and the main south-to-north current establishes itself bringing water from the Bay of Bengal also, the differences noticed earlier tend to disappear. The secondary maxima may also be due to the effect of the influx of new water into the Arabian Sea in this current.

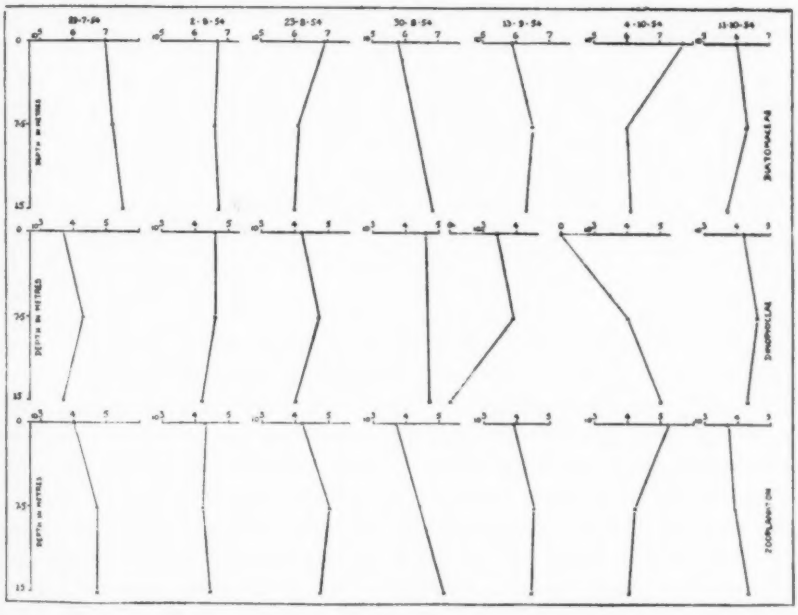
Samples of plankton obtained from Quilandy Bay, 17 miles north of Calicut, was found to be very similar and comparable in general to those of Calicut, as also a few samples examined from Karwar* farther north and Vizhingam* far south. It would appear that the microvegetation of the sea

* Through the courtesy of Mr. S. Ramamurthy and Dr. V. Balakrishnan respectively.

during the south-west monsoon period is more or less uniform in composition over a great extent of the west coast of the Indian Peninsula. There may be some difference as to the time of outburst of the flora depending on the advance of the monsoon from south to north.

X. Vertical Distribution of the Plankton Elements

No detailed work on this aspect could be undertaken; however, some analyses of water samples from known depths taken in a vertical plane (surface, mid-depth and bottom) were carried out and the distribution shown by the Diatomaceæ, Dinophyceæ and the Zooplankton is shown in Text-Fig. 6. Most of the samples relate to the south-west monsoon period.



Text-Fig. 6. Vertical distribution of Diatomaceæ (Bacillariophyceæ), Dinophyceæ and Zooplankton in the water (Explanation in text).

During this period, the Diatoms appear to show a preference to the bottom layers, probably due to the very low salinity obtaining at times at the surface owing to heavy rainfall, either as a reaction to it or to the fall in the specific gravity of the water or both. In the following season, north-east monsoon period, they show a tendency to be distributed in the upper layers. The Dinophyceæ generally seem to prefer the middle layers. It is

also known that the vertical distribution of the Diatomaceæ and Dinophyceæ may be related to their reaction to varying light intensities (Harvey, 1939; Steemann Nielsen, 1939; *cf.* also Phifer, 1934).

The zooplankton is confined to the mid-depths and bottom layers presumably due to their well-known characteristic reaction to light intensity (Russel, 1925, 1926, 1927, 1934 *a*, 1936 *c*; Clarke, 1933; Hardy, 1951; Hardy and Bainbridge, 1954; and Wiborg, 1955). No collections were made after sunset; the above results are based on collections made in the forenoon.

XI. SEASONAL FLUCTUATION IN THE QUANTITY OF THE MAIN CLASSES OF ORGANISMS IN THE PLANKTON

1. *General Remarks*

For studying the fluctuation of the different classes of organisms and of individual species only enumeration data are useful. The Diatoms dominate the flora, followed by the Dinophyceæ and some minor classes of algæ. Under the last, Cyanophyceæ, Silicoflagellatæ, Coccolithineæ and Chrysophyceæ are all included as, either together or separately, they do not contribute to the bulk of the plankton except the Cyanophycean elements, species of *Trichodesmium*, on some occasions during the warmer months, February to April.

The number of species of phytoplankton occurring at a time in any one collection varies from 12–74 and the majority of them are of the Diatoms. The Dinophyceæ constitute the bulk when *Noctiluca miliaris* Sur. happens to occur in large numbers. Nearly 360 species of microscopic plants occur in the plankton on the west coast of India (Subrahmanyan, 1958). The pattern of the flora keeps on changing at varying intervals as also the numbers of the different species. Greatest variety is seen when the plankton content is also higher, but only a few species go to make up the bulk of the flora during the peak periods, sometimes one alone. Further, it is not the same species that contributes to the bulk in all the years or in any two of the years nor is there any constancy in the species contributing to the peaks during the year. A cursory analysis indicates a possible association of certain species during their occurrence similar to the associations seen in the case of land plants and larger benthic sea-weed vegetation. It is likely that there is a sort of mutual antagonism owing to excretion of external metabolites or a conditioning of the medium (Lucas, 1947, 1949, 1955; Rice, 1954) the resultant flora depending on the mutual interaction of the excretions and the degree of tolerance of the organisms to them. The occurrence of different

species in succession also suggests, as mentioned by McCombie (1953, p. 279) in connexion with the lakes of North America, that there is a cyclical succession of pulses of different phytoplankton species throughout the years.

It may be mentioned here, that generally in the inland waters, the phytoplankton organisms inhabiting them—Volvocales, Desmids, and so on, and even some Diatoms—have more or less an annual life-cycle, a period of vegetative phase and then sexual reproduction at the end of which the zygotes formed hibernate for tiding over unfavourable periods such as drought in the warmer parts of the world and severe winter conditions in the temperate and cold regions. The Diatoms—the major constituent of the phytoplankton in all the seas—however, have a life-cycle known to extend over one or more years, even up to 15 years (Hustedt, 1930), but generally a two-to-four-year cycle may be the most usual (Wimpenny, 1936 *b*; 1946, 1956; writer, unpublished). It is proposed to give details of the succession of the species and their peaks, etc., in another paper of this series; however, one instance may be mentioned here, *viz.*, that of *Fragilaria oceanica* Cl. as its fluctuation is referred to in another section. This Diatom, like any other species, multiplies by vegetative division, the cells getting diminished in size after each such division as is characteristic of Diatoms. When the minimum characteristic size (Iyengar and Subrahmanyan, 1944; Subrahmanyan, 1947) for the species is attained, the cells form auxospores, preceded by a sexual process; and, after this, the zygote grows into a large cell (a period of hibernation is not so far known in Diatoms) and thus the characteristic upper limit for the species is attained. This process of auxospore-formation gives the stimulus for renewed rapid multiplication, the rate of which gradually diminishes as the population ages. In the present instance, of *Fragilaria*, it has been inferred from the presence in large numbers of chains composed of large cells approximately every four years or so, that the Diatom has a four-year life-cycle and attains its highest peak of development during those years of auxospore-formation.

The variations in the intensity of the different peaks in the occurrence of phytoplankton are very possibly due to such pulses in the life-history of one or more organisms in the plankton.

Cushing (1956, p. 7) after an evaluation of the density of phytoplankton in the catches made by Savage and Hardy (1935) and Savage and Wimpenny (1936), particularly in relation to *Rhizosolenia styliformis*, for the years 1921–34, found that the conditions that produced very high densities in the concentration of the plankton occurred thrice in twelve years. When this is compared with the observations of Wimpenny on *Rhizosolenia styliformis*

(1936 *b*, 1946) and *Biddulphia sinensis* (1956) where he has found a three- to four-year cycle in auxospore-formation, it is quite evident that these intense pulses mentioned by Cushing (*l.c.*) are a result of auxospore-formation and subsequent intense vegetative multiplication of the species as suggested here for *Fragilaria oceanica* by the writer.

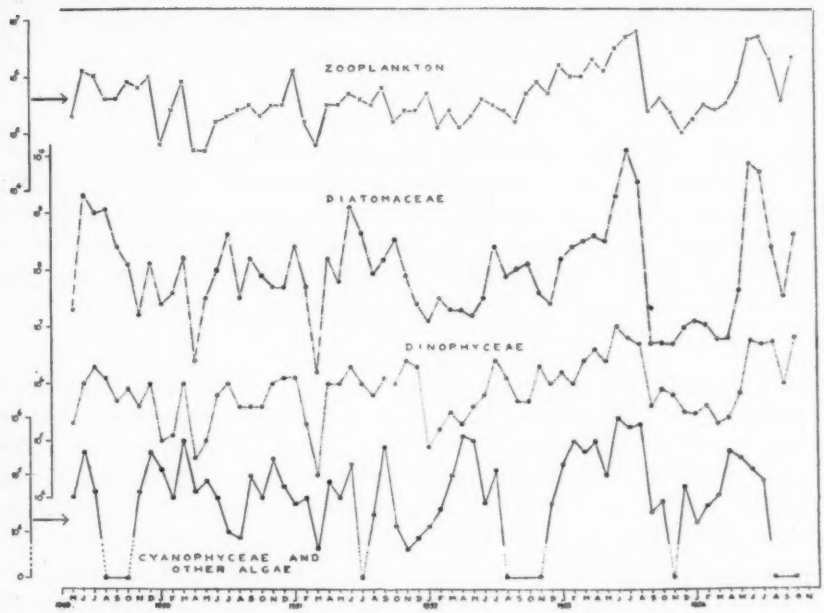
2. *Diatomaceæ*

As the Diatoms constitute the main bulk of the phytoplankton, over 90% by number generally, their seasonal fluctuation resembles that for the total phytoplankton (Fig. 7; compare Table II). They attain the main peak in their occurrence during the south-west monsoon season (June or July) and a secondary peak of occurrence is reached during the north-east monsoon season. In some years there may be one or more subsidiary peaks during both the seasons. The south-west monsoon season appears to be the most favourable one for their growth. During the years of study, the poorest month for Diatoms was March 1951 and the richest, July 1953. In general, the Diatom content of the water was richest in 1953–54, followed by 1949–50, 1951–52, 1952–53 and 1950–51. There appears to be a cyclical change in the intensity of the blooms as was already indicated when dealing with the total phytoplankton, alternate years being somewhat richer than the intervening ones. Further, very intense blooms occur farther apart, after a lapse of a few years of lesser intensity, *e.g.*, 1949–50 and 1953–54. This may (as was seen for *Fragilaria oceanica*) be attributed to the life-history factor of one or more species occurring in bloom in the years concerned. Briefly, the most important genera whose species contribute at times to the bulk production of the phytoplankton are: *Fragilaria*, *Chætoceros*, *Rhizosolenia*, *Coscinodiscus*, *Asterionella*, *Nitzschia*, *Bacteriastrum*, *Thallassiothrix*, *Thallassiosira*, *Actinoptychus*.

3. *Dinophyceæ*

Though the total number of species of Dinophyceæ, recorded as plankton, armoured and naked forms, are nearly as many as those of the Diatomaceæ (Subrahmanyan, 1958) yet, generally, quantitatively they contribute to the bulk of plankton far less than the Diatomaceæ (*cf.* Tables VII, IX and III) except on very rare occasions.

The fluctuation in the quantity of the Dinophyceæ (Text-Fig. 7) is more irregular. This appears to be due to the varied and differing composition of the group, pigmented and unpigmented, armoured and naked, forms occurring at different times, probably governed by different factors. For



TEXT-FIG. 7. Seasonal fluctuation in the quantity (numbers) of Diatomaceæ, Dinophyceæ, Cyanophyceæ, etc., and zooplankton on log. scale (Explanation in text).

example, *Ceratium* spp. are known to react sharply to salinity changes (Graham, 1941 *b*, Graham and Bronikovsky, 1944; Nordli, 1953) and similarly such factors may be active here also. The relationship of some species to environmental factors will be discussed in a later account.

During the south-west monsoon period, the Dinophyceæ occur in large numbers and attain a peak either in June (1951, 1953, 1954) or July (1949, 1950, 1952). At this time, the predominant elements are composed of unarmoured species, pigmented (several species of *Gymnodinium*, *Prorocentrum*, etc.) and unpigmented (species of *Gymnodinium*, *Gyrodinium*, *Noctiluca*, etc.). Among the armoured forms occurring during this season *Dinophysis* spp. and *Ceratium* spp. may be mentioned. *Noctiluca* sometimes constitutes the bulk of the plankton during the season (refer also section of this paper under discoloured water). The armoured forms attain their best development towards the end of the south-west monsoon season or during the following season and one or more peaks of varying intensity occur, at times even surpassing those of the former season (November 1951, October, 1954; Text-Fig. 7). Several species of *Ceratium*, *Peridinium*, *Ornithocercus*, *Dinophysis*,

etc., are noticed during these months. On one occasion, in November 1951, *Ornithocercus magnificus* Stein contributed all the bulk and number of the plankton; but such instances are of infrequent occurrence.

4. *Other Algæ*

Among the other plankton elements met with in the water are species of Silicoflagellatæ, Coccolithineæ, Cyanophyceæ and Chrysophyceæ, very rarely Chlorophyceæ and Euglenineæ. As these occur in very small numbers and infrequently, they have all been combined here and their fluctuation is shown in Text-Fig. 7 and Table III. During certain periods, none of these groups may be present, *e.g.*, August and October 1949, July 1951, August to November 1952, November 1953 and August to October 1954.

During the south-west monsoon season, the Silicoflagellatæ and Chrysophyceæ occur; on some occasions a few of the Coccolithineæ and some of the Cyanophyceæ (*Katagnymene* sp., *Anabæna* sp.) were also present. The same groups are noticed often during the early part of the north-east monsoon months also. From December onwards, species of Cyanophyceæ, chiefly, *Trichodesmium erythræum*, *T. thiebautii*, etc., begin to occur attaining the peak during the warmer months, in February, March or April. These Cyanophyceæ are the only algæ other than Diatoms occurring in noticeable bulk, floating on the surface of the water.

5. *Zooplankton*

Along with the enumeration of the phytoplankton, the faunal elements were also reckoned in the vertical hauls of plankton, primarily to see if there are any relationships present between the two groups. The macro-zooplankton, such as the Medusæ, Salps, etc., are not included, for they are seldom caught in the vertical hauls in appreciable numbers, though at times small Medusæ, Chætognaths and Pteropods occur in such hauls in plenty. The data relate to the total number of all micro-elements, Copepods, larvæ and so on (Text-Fig. 7). George (1953 *a*) has dealt with in some detail the zooplankton of the same region, group-war.

It may be seen that the main peak in the numbers of the zooplankton occur during the south-west monsoon season, in August, immediately after that of the phytoplankton (as seen clearly from the overall average of five years' data). There are one or two more pulses of development taking place in the north-east monsoon season also. In the latter season, though their number is considerably less, their total *bulk* is very high and the size of the individual organisms constituting the bulk also is far larger. Thus, it is seen

that there is a seasonal fluctuation in the occurrence of zooplankton also, as in the case of the phytoplankton, different groups of organisms occurring in succession.

The total number of the zooplankton is considerably less than the Diatomaceæ but approaches the Dinophyceæ as regards abundance in terms of number. During the south-west monsoon period, usually the smaller elements predominate in large numbers, small Copepods, Cladocerans, Tintinnids, and larvæ of several groups; and, during the following season, larger Copepoda, Pteropoda, Polychæte larvæ, Salps, Chætognatha, Medusæ and so on contribute to the bulk, fewer numbers of larger individuals.

XII. The Phenomenon of Discoloured Water

In this phenomenon, only one species is generally involved. The species involved may also vary from year to year or even seasonally. Thus *Noctiluca miliaris* on certain occasions, during June to September, causes a pink discoloration of the sea-water by its high concentration (*see* also Bhimachar and George, 1950); a green discoloration is caused by "*green-Noctiluca*" (*Noctiluca* with a green euglenoid symbiont *Protoeuglena noctilucæ* Subrahmanyan, 1954 *a*) when occurring in enormous numbers as it happened in December 1952 and March 1953; *Hornellia marina* Subrahmanyan (1954 *b*) caused a green discoloration in August and November 1949, September 1952 and September and October 1953; and, a yellow discoloration was caused by a new (unnamed) palmelloid Chrysophyceæ in March 1955. Such occurrences are not a normal feature. Similar phenomena have been recorded from various parts of the world (refer Hays and Austin, 1951 for bibliography; Feinstein *et al.*, 1955; Howell, 1953; Ingle *et* de Sylva, 1955; Margalef, 1956) and on a few occasions elsewhere in India also (Carter, 1858; Hornell, 1917; Subrahmanyan on *Gymnodimium*, unpublished).

It is known that some instances of such outbursts of organisms bring about certain changes in the hydrological conditions (Chew, 1953; Subrahmanyan, 1954 *a*, Prasad and Jayaraman, 1954; Slobodkin, 1953; Ketchum and Keen, 1948; Long, 1953) and sometimes also mortality among organisms in the water including fish due to effect of toxins, presumably liberated into the water (Galtsoff, 1948 *a*, 1948 *b*, 1949; Gunther, 1947; Gunther *et al.*, 1947, 1948; Otterström and Steemann Nielsen, 1939; Stephens, 1948; Shelubsky, 1951; Shilo and Aschner, 1953; Shilo, Aschner and Shilo, 1953; Sommer and Clark, 1946; Sproston, 1946; Subrahmanyan, 1954 *b*, Takano, 1956).

Generally, during the outbursts of these organisms in high concentration, the normal constituents of the plankton are found to be absent or

scarce. It is very likely that this may be due to some deleterious effect produced by their excretions (some visible evidence is available in the case of *Hornellia* (Subrahmanyan, 1954 *b*, pp. 197–98) which resemble " antibiotics " in their action (Lucas, 1949, p. 344; Kylin, 1949). The same explanation may hold good in some instances for the phenomenon of " animal exclusion " postulated by Hardy (1935 *b*, 1936 *a*). More evidence for the antagonistic influence of one organism on another has been recently adduced by Rice (1954; *see* also Pratt, 1943) by culture experiments on *Chlorella* and *Nitzschia*; he concludes that such influences will determine the seasonal fluctuation in the total phytoplankton numbers and of each species, as well as causation of a definite succession of species. Lefevre *et al.* (1949, 1952) have also obtained similar results as those of Rice (*cf.* also Davis, 1954 *b*).

XIII. SEASONAL FLUCTUATION OF THE PHYTOPLANKTON AND ZOOPLANKTON COMPARED WITH EARLIER OBSERVATIONS

1. *Phytoplankton*

It is well established that in all the temperate and colder regions of the world, there is a rhythm both in the qualitative and quantitative distribution of the plankton which is repeated every year though with slight variation in some years. Generally, there occurs a main bloom in the spring and another of lesser intensity in the autumn. This has been found to be so both in the northern and southern hemispheres.† There are reports of several expeditions and cruises which give particulars relating to plankton conditions in these waters, lending support to the general picture obtained by the other individual investigations. According to Allen (1934) the seasonal fluctuation is not so marked at stations in southern California waters as they are in those farther north. Perhaps, in the lower latitudes, the seasons lose their entities and hence such sharp fluctuations characteristic of higher latitudes are not evident nearer the Equator. Unfortunately, as mentioned already in the beginning, we have only very little information for areas in the warmer regions of the world and even these cover only short periods permitting no definite conclusions; a few of them, however, throw some light on the relative standing crops of the regions in the temperate and tropical zones (Allen and Cupp, 1935; Allen, 1924, 1939; Gilson, 1937; Graham, 1941 *a*, Riley, 1938, 1939; Riley, Stommel and Bumpus, 1949).

The accounts for the warmer parts of the world, where there have been some continuity of observations, may be briefly cited here.

† Owing to paucity of space, a list of references on this aspect is omitted here.

One of the earliest attempts to study this aspect in a warmer region of the world appears to be by Hornell and Nayudu (1923) who studied the plankton on the west coast of India at Calicut in connexion with their work on the life-history of the Indian oil-sardine, *Sardinella longiceps* Cuv. *et* Val. They speak of an outburst of Diatom bloom in May, which fell somewhat in June and reached the maximum in July, and fell off again to a minimum in December. There were small pulses of abundance in January and February, a fall in March and a rise in April.

According to Menon (1945), on the Trivandrum coast, farther south of Calicut, the Diatoms gradually increase from January to a maximum in May and there is no clear secondary maximum. More work continuously for a number of years is needed here for a clear elucidation of the cycle.

In the Bombay harbour, one year's investigation indicated that phytoplankton was scarce from May till August after which there was an increase till the maximum was reached in January or February (Gonzalves, 1947). The cycle noted here is, very probably, influenced by the peculiar environment of a harbour; it is possible, that the cycle of phytoplankton bloom in the sea off Bombay beyond the harbour pollution might be more in line with that described by the writer in this account.

At Calicut, according to Chidambaram and Menon (1945), who had no data for the crucial south-west monsoon months of June, July and August, the volume of plankton was at its maximum in September; there was a fall in October and November, a slight rise in December, and scarcity in January following. From February to May, Plankton production was more or less steady with minor fluctuations occasionally.

George (1953 *a*) made a general study of the plankton at Calicut for about three years. He found the occurrence of the peak period for phytoplankton in the south-west monsoon months. A second peak may occur in December or January. In general our observations are in agreement.

Coming to the east coast of India, Menon (1931), who investigated the plankton for two years at Madras, found that the Diatom maximum occurs in April–May. There is a slight fall in June, a rise in July and a rapid fall thereafter. A secondary maximum occurs in December. Further, according to him (*l.c.*, pp. 493–94) the general maximum is a culmination of a regular and constant Diatom increase beginning in September unlike the European waters where it is a sudden rise beginning in March. The Peridiniales also attain their maximum in May.

Ramamurthy (1953 *b*) who made study of the plankton at Madras over 20 years after Menon, for about 14 months, noticed a difference in that while August–September were months of scarcity for Diatoms in Menon's records, they were not so in his records. According to him, February, April, May, August, September, November and December were rich months while January, March, June, July and October were poor months.

At Waltair (farther north of Madras) Ganapati and Rao (1953) and Ganapati and Murthy (1955), in studies lasting for a year in each instance, report a spring maximum extending from February to April and an autumn maximum from October to December (Ganapati and Rao) and November (Ganapati and Murthy).

On the south-east coast of the Indian Peninsula, at Krusadai in the Gulf of Mannar, Chacko (1950) states that the Diatom maximum is usually from June to November. In the same Gulf, in a study covering two years, 1950 and 1951, according to Prasad (1954 *a*) the phytoplankton cycle shows more than one maximum during the year; a peak in February–March due to local flowering of a single species and a summer, August–November, peak constituted by several species of Diatoms. In his further study, extending from July 1951 to June 1953, the same author (Prasad, 1956) found at a Gulf of Mannar Station, three distinct blooms of phytoplankton, in January, April–May and October–November. In the Palk Bay station, the magnitude of the crop was of a higher order and two peaks of production occurred, one single prominent one during the summer months April–June, and another in October–November.

There are some accounts available from other warmer areas of the world which may be of interest here. In the Great Barrier Reef region, Marshall (1933) could not establish a real seasonal fluctuation for the phytoplankton; moreover, the period of investigation extended only for a year there.

Haneda (1942) found in Palau Island in the tropical Pacific periods of maximum and minimum in the plankton content of water.

Kow (1953) found in the Singapore Straits, almost on the Equator, the main bloom of phytoplankton occurring in April or May; and there were peaks, one or more, of varying intensity at other times of the year.

The results of the present investigation at Calicut on the west coast of India, covering a period of over 5 years, clearly shows that there is a seasonal variation in the abundance and occurrence of the phytoplankton organisms which recurs year after year, though with slight variation in the intensity of

the bloom and its time of occurrence, even as has been observed in the temperate and colder seas. The maxima occur during the south-west monsoon period, in June or July. In some years there is a sharp fall in the quantity of phytoplankton in August, very probably due to the grazing effect of the zooplankton which attain their primary maxima about that time. The phytoplankton is poorest generally in the month of November and one or more pulses of production occur during the north-east monsoon months, between December and April. Thus, in the Indian waters also, it may be stated with certainty that there is a double or bimodal oscillation in the fluctuation of the standing crop of phytoplankton; however, the period of occurrence of the bloom varies from place to place somewhat and there is no general uniformity as seen in the temperate regions. The factors responsible for the production of phytoplankton and for the variation in the timing of the bloom will be discussed elsewhere (Part II).

2. *Zooplankton*

As in the case of phytoplankton, the fluctuation in the abundance of zooplankton has been thoroughly investigated in the temperate and colder seas both in the northern and southern hemispheres. It has been found that there is a pronounced maximum in late spring or summer and a minimum in the winter and occasional pulses of abundance sometimes at other periods in between (*e.g.*, Harvey *et al.*, 1935; Russel, 1936 *c*; Johnstone, Scott and Chadwick, 1924; Wiborg, 1944, 1954; Deevy, 1952 *a, b*; Dakin Colefax, 1940; Sheard, 1949). Data for the tropical and sub-tropical regions of the world are indeed few as there have been no continuous investigations in these regions. The accounts that are available confirm the presence of a seasonal fluctuation reported in the present account.

Menon (1931) found the period of abundance of the zooplankton in the Madras waters between November and February. On the Trivandrum coast (Menon, 1945) the data for zooplankton show no clear peaks of abundance, though December to February in 1938–39 and February to May in 1939–40 may be considered as having a high zooplankton content; in the former year, the zooplankton content was of a higher order. In the Gulf of Mannar near Mandapam, in a study covering 1950 and 1951, Prasad (1954 *a*) found a bimodal cycle for the zooplankton, with one peak in February–April and the maximum peak in October, with a minimum in July. In a later account the same author (Prasad, 1956) found a bimodal cycle both in the Gulf of Mannar and the Palk Bay, the two stations, however, differing in the pattern of the cycle; while one station showed abundance, in the other the zooplankton was poor and *vice versa*.

In the Great Barrier Reef, Russel and Coleman (1934) could find no sharp fluctuation in the abundance of the zooplankton as in the temperate regions; but, the quantity did show a fluctuation at different seasons and as the period of study was only one year, it could not be stated whether the cycle would repeat itself. King and Demond (1953) and King and Hida (1954) were able to find a seasonal abundance in the zooplankton content of the water in the Central Pacific and Hawaiian waters respectively. Kow's (1953) data for the Singapore Straits suggest a possible seasonal fluctuation as shown by the abundance of the Copepods which form over 70% of the zooplankton population.

In the present area, the main peak in the numbers of zooplankton occurs during the south-west monsoon season, in August, immediately after that of the phytoplankton (as reckoned from the average of 5 years' data). There are one or more pulses of development during the following north-east monsoon season. As already mentioned, the zooplankton exceeds the phytoplankton in bulk on most of the occasions in the latter season though numerically fewer. As in the case of the phytoplankton, although the results of all the investigations agree in the record of a seasonal abundance in all the places investigated, differences occur relating to the time of their peak development and the intensity of the crop.

XIV. PHYTOPLANKTON-ZOOPLANKTON RELATIONSHIP

It is well known that zooplankters depend on phytoplankton for food, and naturally, some relationship is to be expected between them and their respective distribution. This relationship has been dealt with and reviewed by many workers in the past, mostly based on work in the temperate and polar waters (Harvey, 1934 *b*; Harvey *et al.*, 1935; Bigelow, Lillick and Sears, 1940; Mare, 1940; Hart, 1942; Gauld, 1950; Marshall, Nicolls and Orr, 1934; Marshall and Orr, 1952; Marshall, 1949; Barnes and Barnes, 1956; Wimpenny, 1936 *a*; Hardy, 1935 *b*; Lucas, 1936 *a*; 1947, 1949 and 1955; Steemann Nielsen, 1937 *a*; King and Demond, 1953; King and Hida, 1954; Clarke, 1939; Riley, 1946; Fleming, 1939 and Bainbridge, 1953). The substance of these investigations indicates that an abundance of phytoplankton leads to an increased development of zooplankton and the relationship between them is *direct* and the *inverse* relationship at times recorded is caused by the grazing down of the phytoplankton population by the zooplankton. Further, it was also held that the inverse relationship was brought about by the animals avoiding areas rich in phytoplankton (Hardy, 1935 *b*; Lucas, 1936 *a*) presumably owing to the effect of external

metabolites of the plant population which adversely condition the medium (Lucas, 1947, 1949 and 1955).

In the following brief account some of the relevant facts emerging from the foregoing investigations are discussed with reference to observations made here, besides referring to one or two recent papers.

An extensive experimental study of the relationship between phyto- and zooplankton by Bainbridge (1953) has shown that the inverse distribution met with may be accounted for by a combination of migration and grazing and the exclusion mechanism as a means of producing the inverse relationship is operative only in instances of monospecific blooms of toxic flagellates, such as *Gonyaulax*.

Kow (1953) found a direct relation between phytoplankton and Copepods in that their peak of occurrence coincided.

In the Indian waters, Prasad (1956) found at a station in the Gulf of Mannar that the relation between phytoplankton and zooplankton was inverse, whereas at a station in the Palk Bay, the relationship was direct. The Copepod-Diatom relationship was of a similar nature at the respective stations (*see* also Prasad, 1954 *b*).

The present investigation indicates that in the study of phyto-zooplankton relationhsips, the time factor or seasonal factor, has also to be taken into consideration. It was pointed out earlier that in terms of the *bulk* of the standing crop, the biological year shows two clear periods, (i) the predominantly phytoplankton period, the south-west monsoon season, May to September–October, and (ii) the predominantly zooplankton period, the north-east monsoon months, October–November to April following.

The data collected during the period of five years show that the number of zooplankton elements tends to increase gradually with the increase of the phytoplankton elements (number and pigment units) from the beginning of the south-west monsoon season. This may be seen from a casual examination of Table XIII, in which the five years' data are averaged (*cf.* also Tables II and III; and also Text-Fig. 7). The zooplankton (in terms of *number*) attains its peak of development at the same time as or immediately following the peak in the phytoplankton production. The trend of increase of both indicating a " direct " relationship is unmistakable though study of individual samples may not show a " proportionate " increase between them, this being due to the changes in the composition of the flora and fauna alike. On certain occasions, when the phytoplankton is composed of mainly setoid

TABLE XIII

Phytoplankton-Zooplankton relationship

Month	Zooplankton number	Phytoplankton number × 1,000,000	Phytoplankton Plant pigment units (Harvey)	Remarks
May ..	403,000	80	2,204	
June ..	1,143,000	1,071	3,684	1. The figures given are the average of five years' data
July ..	1,357,000	3,405	8,002	
August ..	1,393,000	971	3,782	
September ..	366,000	134	3,020	2. The Dinophyceæ including *Noctiluca* have been reckoned under phytoplankton
October ..	413,000	134	2,342	
November ..	459,000	25	1,412	
December ..	430,000	48	1,512	
January ..	741,000	100	1,276	
February ..	382,000	71	1,516	
March ..	518,000	104	1,370	
April ..	610,000	115	1,370	

forms like *Chætoceros* spp., *Bacteriastrum* spp., *Rhizosolenia* spp., etc., a fall in the number of zooplankton, somewhat proportionate, becomes evident which might be interpreted according to Hardy as " animal exclusion " owing to a deleterious influence exercised by these forms on the faunal elements leading to a patchy distribution of the plankton. Plenty of examples of patchy distribution are also available from the Antarctic and the seas around the British Isles (Lucas, 1936 *b*, 1940; Lucas and Macnae, 1941; Hardy, 1935 *a*, *b*; 1936 *a*, *b*; 1955; Hart, 1934; Savage, 1930; Savage and Hardy, 1935; Savage and Wimpenny, 1936; Wimpenny, 1936 *a*; Rae and Fraser, 1941). In contrast, when the plant forms involved are non-setoid, *e.g.*, species of *Coscinodiscus*, *Thalassiosira*, *Fragilaria*, and so on, a proportionate increase of animals is noticed. In general, during this season, the relationship between phyto- and zoo-plankton appears to be the same as recorded by Marshall, Nicolls and Orr (1934) and Steemann Nielsen (1937 *a*), *viz.*, a direct one. This is supported by the presence in the plankton samples of developmental stages of many groups of animals in considerable numbers. In terms of total *bulk*, the zooplankton during this season is no match to the phytoplankton *bulk*, the conditions for the production of the latter being at its optimum. Most of the animals are small-sized forms; nevertheless, the trend of their increase shows that they feed and reproduce on account of the increased availability of plant food. Among the zooplankton organisms met with during the season are Foraminifera, Radiolaria, Tintinnids, Polychæte larvæ, larval Bivalves, Cladocerans, small Copepods, Nauplii, Amphipods, Lamellibranch larvæ and towards the end of the period, Salps, Tunicates and Prawn larvæ, also (*see* also George, 1953 *a*).

During the north-east monsoon season, the general picture of the standing crop is very different. Except on a few occasions, when the phytoplankton bloom is at its second peak of production, zooplankton is seen *prima facie* to dominate the *bulk* of the standing crop. The majority of the zooplankton forms are larger in size compared with those of the earlier season; and it may be stated here that the Copepod element is the dominant faunal form in the plankton and of utmost importance in the economy of the sea as it is elsewhere also. Besides the Copepods, the forms occurring include Salps, Medusæ of varying sizes, Ctenophores, Chætognaths, Decapod and Molluscan larvæ and so on (*cf.* also George, 1953 *a*). However, the relationship seen in terms of *number*, direct increase in the number of zooplankton elements with an increase in the *number* of phytoplankton, is also seen during this season except on a few rare occasions as on November 7th, 1949, March 12th and 19th, 1951, and April 2nd, 1951, when even the number of zooplankters were above or equal to that of the phytoplankton, which obviously

indicates that the production rate of phytoplankton has declined and/or grazing rate has increased, resulting in an inverse relationship such as that mentioned by Steemann Nielsen (*l.c.*). Such a situation lends support to the view propounded by Fleming (1939) also. Moreover, it is seen that the rate of fall in the production of phytoplankton during the north-east monsoon season as noticed in terms of the magnitude of the standing crop is of a higher order than the rate of fall in the number of zooplankton during the same period; on the other hand, the *quantity* of animal matter has increased considerably, obviously at the expense of the phytoplankton. The conspicuous fall in the quantity of phytoplankton in November generally (Text-Fig. 4) is very probably due to the heavy grazing by the increasing number of larger zooplankton organisms and this accords with the suggestion of Harvey (1934 *b*) and Harvey *et al.* (1935) for the English Channel.

Data relating to the dry weight estimations of the standing crop, the *biomass*, throw some interesting light on the interrelationships of the phyto- and zoo-plankton community. It was pointed out earlier that the sharp fluctuation seen, when the standing crop is reckoned in terms of number and pigment units, is not seen when the same is viewed with data relating to dry weight estimations; and, further, the overall ratio of the crop between the two seasons, a predominantly phytoplankton one and the following predominantly zooplankton one, shows a close approximation indicating a balance in the biological content of the water as between plant and animal life. This seems to indicate that the relationship between these two forms of life, animal and plant, in the sea, is not one that can be ascertained by the present methods of the estimations of the standing crop. The relationship is one that involves a large number of factors, the rate of increase of plant matter, the rate of grazing by animals, the quality of food consumed by the animals, mode of feeding—indiscriminate or selective—and hydrological conditions affecting the biological elements. The qualitative nature of the flora and fauna changes frequently; it is possible that certain animals are associated with a particular type of vegetation and so on. Too much emphasis appears to have been placed on the *number* factor in judging the relationship of the phyto- and zoo-plankton in the past, of course for want of a better method. The *bulk* also needs to be considered as may be seen from the present investigation. Further, in experimental investigations, the species employed, particularly phytoplankton ones, are those that grow easily under laboratory conditions; these forms are not found generally in quantity in nature. Inability to reckon all these may, to some extent, explain the slight variations in the interpretations put forward of observations from nature.

The relationship between phyto- and zoo-plankton as it obtains here, therefore, may be generally considered to be *direct* in terms of *number* of organisms and *bulk* during the south-west monsoon season; and direct in terms of *number* but inverse in terms of *bulk* during most of the north-east monsoon season. It is not possible to say how exactly this relationship is brought about in these waters in the absence of information on several aspects; it is possibly brought about both by the vertical and horizontal migration of the faunal elements as suggested by Bainbridge (1953) and, from the presence of developmental stages, it may be stated that a good number grows and thrives *in situ* also among the same plankton environment, even in dense patches of phytoplankton.

Sometimes, during the south-west monsoon season, when there is heavy production of phytoplankton, surface plankton catches were found to consist of mainly zooplankton—Copepods and Cladocerans predominantly—while bottom samples of plankton were mainly of phytoplankton with some zooplankton. On such occasions, it has been noticed that the salinity of the surface water was very low, even below 20‰ whereas water at the depth of 15 metres showed a salinity of *circa* 30‰. Further, on many such occasions, the phytoplankton also was composed mainly of setoid forms; mention of the possibility of such a reaction of the fauna to such floral elements has already been referred to. It is possible that salinity also may have a relation connected with this reaction; the Diatoms may have sunk owing to the very low specific gravity of the surface water and, as their concentrations may have increased so much in the bottom layers, some zooplankton may have migrated and aggregated at the surface as they could, probably, tolerate a much lower salinity. Obviously, the phytoplankton could not, for the specimens seen at the surface were mostly dead.

Before concluding this section, the "animal exclusion" hypothesis of Hardy, mentioned earlier, may be examined in relation to the occurrences here. According to Bainbridge (1953) this operates in areas where there are monospecific blooms of a toxic flagellate. Some observations on the plankton of the west coast of India lend support to this view of Bainbridge. Sometimes blooms of green *Noctiluca* (*Noctiluca* with the symbiont *Prototeuglena noctilucæ* Subrahmanyan, 1954 *a*) occur in the waters here and also on the south-east coast of India (Prasad, 1953; Prasad and Jayaraman, 1954). On the west coast, whenever the concentration of this form was so high as to discolour the water, it was found that virtually no other organisms were present in the water, whether Diatoms or zooplankton. This has been observed on the east coast also, where, in addition, it was noticed that the

" Choodai " (*Sardinella* spp.) become scarce when this *Noctiluca* occurred there (Prasad, 1953). Again, during the occurrence in high concentration of this *Noctiluca* certain changes in the hydrological conditions have been observed, particularly related to the silicate content of the water (Subrahmanyan, 1954 *a*; Prasad and Jayaraman, 1954). This aspect is discussed by the writer later below (Part II). It may be mentioned here that elsewhere also hydrological changes have been noticed connected with such blooms, *e.g.*, the " Red Tide " on the Florida coast (Chew, 1953; Slobodkin, 1953; Ketchum and Keen, 1948; Long, 1953).

The writer would like to point out here that no deleterious effect appears on the plankton organisms or fishes when the common pink *Noctiluca miliaris* Sur. occurs, even in high concentrations, discolouring the water pink. Often this takes place during the south-west monsoon season on the west coast of India and at such times large quantities of phytoplankton as well as zooplankton also occur along with it. However, it may be mentioned here that Aiyar (1936) has reported mortality of fishes on the Madras coast, and Bhimachar and George (1950) also report a set-back to the fishery on the west coast during the occurrence of this *Noctiluca* (*cf.* however Subrahmanyan, 1954 *b*, pp. 188–97).

Again, on the west coast, *Hornellia marina* Subrahmanyan, a Chloromonadine flagellate, when occurring in sufficiently large quantities as to discolour the water green, appears to have a deleterious effect on all other plankton organisms and often causes fish mortality (Subrahmanyan, 1954 *b*). The extrusion of a certain substance seen in this form at times (Subrahmanyan, *l.c.*, p. 197) presumably brings about a toxic condition in the water for other organisms.

The other instance of a monospecific bloom was when an unnamed Chrysophycean palmelloid alga (Subrahmanyan, unpublished) occurred here discolouring the sea-water somewhat yellow. Most of the usual organisms in the plankton were absent except an odd Diatom here and there.

In a series of articles, Lucas (1936 *a*, 1947, 1949, 1955) has discussed and pointed out the possibility of a non-predatory relationship between the phyto- and zoo-plankton, especially, brought about by *ectocrines* (external metabolites having important environmental significance) which range from toxins to vitamins and hormones in their ecology and which might lead to a biological conditioning of the water by the organism or organisms which precede. It is very possible that this type of relationship becomes pronounced only when a monospecific bloom occurs. These observations made on the west coast of India would appear to lend some support to the statements of Lucas

mentioned above and of Rice (1954) explaining mutual antagonisms between certain organisms.

In the literature, one finds mostly some peridinians—*Gymnodinium* spp., *Gonyaulax* spp., etc., associated with the type of phenomena described above (Hays and Austin, 1951; Galstoff, 1948 *a*, *b*, 1949; Davis, 1948; Allen, 1933, 1940, 1942 *a*, 1942 *b*, 1943, 1946; Santos-Pinto, 1949; Hornell, 1917). So far, the organisms described here have not been reported and, therefore, are of special interest.

The researches in the North Sea on the Diatom and zooplankton patches and the relationship of the same to the herring are of interest in this connexion (Pearcy, 1885; Bullen, 1908; Hardy, 1924; 1926, 1936 *a*, *b*; Savage and Hardy, 1935; Savage, 1930, 1932; Savage and Wimpenny, 1936; Lucas, 1936 *b*, 1940, 1942; Lucas and Macnae, 1941; Lucas, Marshall and Rees, 1942; Henderson, Lucas and Fraser, 1936; Henderson, 1936). However, in a recent contribution, Cushing (1956), after special observations on this aspect of exclusion, comes to the conclusion that these instances of " exclusion " in the North Sea are not of general occurrence at the concentrations of the Diatoms in the patches met with and the earlier conclusions of the authors were due to some discrepancies in the timing of the investigations. Much higher concentration would be required to produce such an " exclusion ". Bainbridge's (1953) conclusion on such occurrences, as also that of the writer already cited, would appear to support Cushing's view.

XV. Relationship between Plankton Production and Fisheries

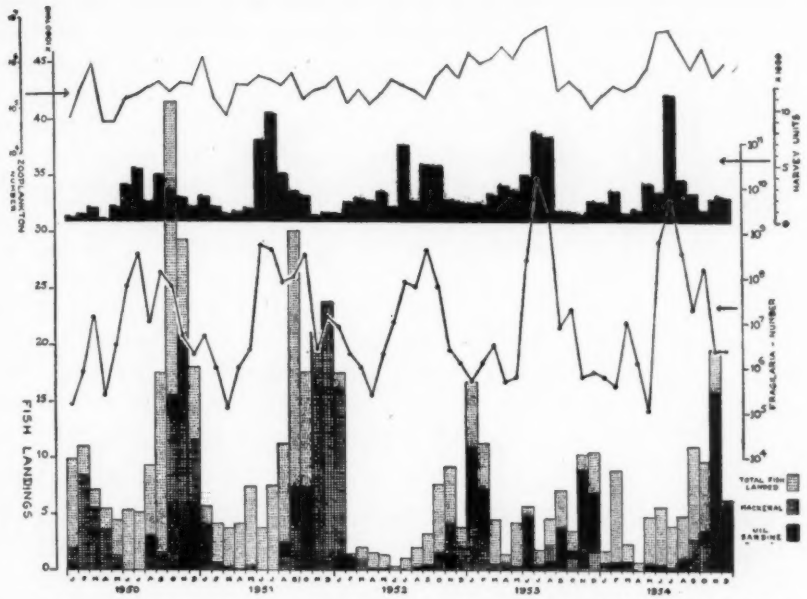
The object of fishery research is mainly to devise a method for the forecasting and controlling of future fish supplies. As the phytoplankton elements, the main synthesizers of food in the sea, fluctuate in relation to so many environmental factors, they form an important and convenient basis in assessing the fluctuations of the stock of edible fish. It is well known that a rich phytoplankton crop leads to a rich zooplankton crop and good survival of young fish (Russel, 1936 *b*; Harvey, 1950). Delsman (1939) states: " Where no rich plankton develops, no rich macrofauna, no abundant fish population can either be expected." Hesse *et al.* (1951) state that " the dependence of various elements of the food chain on a preceding one conditions the distribution of the larger forms". By means of experiments carried out by application of inorganic fertilizers to ponds, Smith and Swingle (1939) have clearly demonstrated that increased plankton production increases fish production and a direct relationship between average production of plankton and production of fish exists, though no direct relationship was evident

between zooplankton alone and fish production (*cf.* also Tal and Shelubsky, 1952). The experiments carried out for marine fish cultivation also by artificial fertilization of sea lochs in Scotland bring out the rôle of plankton in the production of fish; the growth of fish was greater owing to increased supply of food consequent on increased production of plankton (Gross *et al.*, 1944, 1946; Gross, 1947, *a*, *b*, 1949; 1949 (50), *a*, *b*; Raymont, 1947, *a*, *b*, 1949, 1950; Marshall, 1947; Marshall and Orr, 1948; Orr, 1947; Gauld, 1950). As Lewis (1929, p. 156) has observed " by learning the relation of the fish to its food environment, it may become possible by examinations of fluctuations in the quantity of fish food, to evaluate the significance of one of those factors that determine in advance the relative abundance of fish for certain year classes. It may also become possible to determine the cause for the apparent migration of certain fish".

The Indian oil-sardine, *Sardinella longiceps* Cuv. *et* Val., and the Indian mackerel, *Rastrilliger kanagurta* Cuv., constitute the bulk of the fishery resources of the west coast of India and quite a considerable number of the people of the region is dependent on these fisheries for their livelihood. Nair and Chidambaram (1951) have discussed the magnitude and importance of the oil-sardine fishery in the economic life of the people of the west coast and later Nair (1952) has discussed the revival of this fishery after a long period of lean years. Pradhan (1956) has reviewed the mackerel fishery of Karwar, the most important centre for this fish on the west coast. The other fishery resources are contributed by prawns, white sardines, the flat fish, *Cynoglossus*, etc. (*cf.* also Panikkar, 1952; Rao and Panikkar, 1949).

In Text-Fig. 8, the total landings of fish for an extent of the west coast of India, about 150 miles of the coast-line, north of Ponnani to Mangalore (Zone 9 of the Central Fisheries classification), is graphically shown as also the landings of the oil-sardine and the mackerel. In the same figure, phytoplankton data are represented as Harvey Units, and total zooplankton data and that for *Fragilaria oceanica* Cleve in numbers. The general trend of the fluctuation of fish landings shows that the quantity landed goes up following the bloom of phytoplankton or even, sometimes, during the height of the bloom as in June 1953. The fish landings at Calicut also reflect the same trend as Zone 9 mentioned above and hence is not treated separately.* A clearer appreciation of the relationship between plankton production and fisheries is possible only if the fishes concerned are considered in relation to their food habits and the constituents in the plankton. Therefore, the present

* My thanks are due to Mr. P. K. Jacob for supplying me with data relating to landings of the oil-sardine and mackerel at Calicut collected by the Madras State Fisheries Department.



TEXT-FIG. 8. Seasonal fluctuation in the quantity of phytoplankton, zooplankton and *Fragilaria oceanica* in relation to the total commercial fish landings and of the mackerel and oil-sardine on the west coast (Explanation in text).

knowledge we have on the oil-sardine and mackerel are considered here with reference to plankton data and compared with similar observations elsewhere to explain how such relationships are brought about.

The oil-sardine fishery.—In the course of his investigation on the oil-sardine, Nair (1952) established that the oil-sardine is a plankton feeder, feeds mainly on phytoplankton and shows a preference to the Diatom, *Fragilaria oceanica* Cleve. According to Subrahmanyan (unpublished), this Diatom has a life-span of three to four years, during which the quantity of this Diatom in the plankton fluctuates, there being outstanding peaks of production at intervals at about 4 years or so brought about by its mode of reproduction. Similar cycles of abundance have been recorded for *Rhizosolenia styliformis* and *Biddulphia sinensis* in the North Sea (Wimpenny, 1936 *b*, 1946, 1956; Cushing, 1956). A study of the fluctuation in the quantity of this Diatom, *Fragilaria*, and the oil-sardine landings led to a short account by Nair and Subrahmanyan (1955) in which it was pointed out that the bloom of this Diatom could be used as an indicator of the abundance of the oil-sardine.

In Text-Fig. 8, the quantitative fluctuation of *Fragilaria* during the years 1950–54 and also the oil-sardine landings are depicted. The oil-sardine fishery commences with the commencement of the *Fragilaria*-bloom in the waters here. It would appear that as more and more sardines invade the waters (as evidenced by increasing landings of fish) this plankton is grazed down and becomes apparently almost depleted. The grazing activity would seem presumably to start at the periphery of the phytoplankton patch during the early part of the season and then gradually extend inward until finally the bloom is grazed down. (It may be mentioned here, that during the early part of the season, fishing activity is very much limited because of the inclement weather conditions and, consequently, full exploitation of the fishery during this season does not take place. This point is referred to later below.) Savage and Wimpenny (1936) have observed a massing of both zooplankton and the herring on the edges of the Diatom patches in the southern North Sea and great intensity of fishing takes place along the edge of the phytoplankton patch.

After the main peak of the fishery has been attained and passed following the main bloom of *Fragilaria*, it has been found, on some occasions, that if an increase of this Diatom occurred in the waters, sardine also became available and once again the Diatom is grazed down along with some other elements. Even when *Fragilaria* is apparently absent in the usual plankton catches, gut contents of sardines have shown *Fragilaria* in not inconsiderable numbers; the scarcity of this Diatom in the water may, perhaps, be due to the grazing effect on it by the fish. Therefore, it seems probable, that the relationship seen between this phytoplankton element and the oil-sardine is somewhat similar to that seen in the instance of phyto- and zoo-plankton relationship, *viz.*, a direct one to start with which becomes inverse owing to the grazing rate exceeding the multiplication rate of the Diatom.

Obviously, this relationship leads to the query whether one could expect increased oil-sardine catches based on the intensity of the monsoon bloom of *Fragilaria*; in other words, could the intensity of the bloom of *Fragilaria* be an indication of the prospects of the oil-sardine fishery of the following seasons? Nair and Subrahmanyan (1955) adduced some evidence which indicated that an increase in the abundance of this diatom could mean increased oil-sardine catches. Records show (Subrahmanyan) that between the years 1949 and 1954, *Fragilaria* had two outstanding peaks of production, in 1949 and 1953 and it was surmised that the bloom of 1949 helped to revive the fishery leading to increased landings in 1951–52 compared with earlier years, which culminated in the outstanding landings of sardines for severa

decades in 1953, in which year *Fragilaria* also showed an outstanding peak of production (Text-Fig. 8).*

The relationship mentioned above between the oil-sardine and *Fragilaria* naturally raises the question of the feeding habits of this fish. The observations (Nair, 1952) show that it is not *exclusively* dependent on *Fragilaria* but only appears to show a *preference* for it. Many other species of Diatoms as well as other organisms have been reported from its gut content. In the absence of a detailed quantitative study of the gut contents in comparison with the plankton content of the water, it is not possible to come to any conclusion on this subject. However, the observations on the California sardine are of interest here. Lewis (1929) found that Diatoms and Dinoflagellates are used chiefly as food by this sardine though, occasionally, Copepods also are eaten; a definite relation exists between numbers and species present in the digestive tracts of the sardines and in the surface plankton about them. His observations would indicate that there is some selective intake of food. This view of Lewis's is disputed by Parr (1930) who states that ingestion of phytoplankton is incidental and zooplankton forms are the real objects of special pursuit. Radovich (1952) after the analysis of the food of the same sardine, *Sardinops cærulea*, came to the conclusion that this form is a particulate as well as a filter feeder (also *Cal. Co-op. Sardine Res. Program*, 1952, p. 24). The presence in the gut of the oil-sardine here of several other species from the plankton and the absence of *Fragilaria* on some occasions (when this Diatom is not present in the water obviously) would indicate that the large quantities of *Fragilaria* when present is due to a *particulate* form of intake of food.

The mackerel fishery.—Studies made so far on the feeding habits of the mackerel show that the mackerel is an omnivore (John and Menon, 1942; Chidambaram, 1944; Bhimachar and George, 1952). Zooplankton, particularly, Copepods, constitute the bulk of its food. It may be mentioned that Copepods generally are the dominant elements of the zooplankton.

Discussing the correlation between mackerel food and the plankton, Bhimachar and George (1952) state that the composition of the plankton in the water is reflected in the gut content of the mackerel except for certain "non-edible" forms like *Noctiluca*, Salps, Medusæ and so on, and food composition varies from season to season. A somewhat similar relationship

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* The next outstanding bloom for *Fragilaria* was expected in 1957 south-west monsoon (writer's calculation); this has come true as also a very good oil-sardine fishery during the 1957–58 fishing season.

has been found by Kow (1950) in his observations on the food habits of the fishes of Singapore Straits. These accounts would indicate that the mackerel concerned is not quite discriminate in its mode of intake of food except to the extent of " avoiding " certain forms (*cf.* Pradhan, 1956, pp. 168–71, also).

The fluctuation of the mackerel fishery also is shown in Text-Fig. 8. Though no such close relationship between the mackerel and any other factor is evident as was found between *Fragilaria* and oil-sardine, it may be seen that there is, to some extent, a direct relationship to start with between phyto- and zoo-plankton on the one hand, and mackerel on the other which, later on, as the consumption of plankton increases, becomes inverse. It may be seen from the figure that mackerel catches are of a higher order when the standing crop of zooplankton is lower; in other words, zooplankton crop never reaches a high peak when mackerel is present. Until more is known about the feeding habits of the mackerel and of the quantitative composition of the food ingested, and the cycle of occurrence of the several organisms constituting its food and their relative abundance in the water, no indicator organisms can be specifically recognized.

As there is not much information available about the food of several other fishes of commercial importance on the west coast of India no further attempt at correlation is possible now (*cf.* however Seshappa and Bhimachar, 1955). However, it may be mentioned that considerable quantities of prawn are landed here which also form an important fishery during the months following the main outburst of phytoplankton. The food of the prawn also consists of considerable quantities of phytoplankton elements, particularly *Fragilaria*, *Coscinodiscus*, *Pleurosigma*, *Navicula*, *Cyclotella*, etc. (Menon, 1951), on some of which the prawn feeds when the elements sink to the bottom while others feed at the bottom. The food of *Kowala coval* (Cuv.), the white sardine, also consists of plenty of phytoplankton elements (*see* Nair, 1951, Table I). It may be noted that the peak of the fishing season succeeds the main production of phytoplankton on this coast.

It may be pointed out that with the commencement of the south-west monsoon, owing to the stormy weather, regular fishing operations are at a standstill, till calmer conditions prevail in the sea, about August. So, absence of fish landings during this early part of the period may not be entirely due to the fishes being absent from the waters, but absence of fishing operations. It may be seen from the data that good landings can be expected even during the peak period of the monsoon as may be seen from the landings made in June 1953, when the fish mainly caught was the oil-sardine, a phytoplankton feeder (Text-Fig. 8). Fishing operations are possible during this season in

some years, when mud bank formation occurs along this coast, as it did in 1953. During the occurrence of mud banks, as mentioned earlier, the sea is calm in its environs and fishermen are able to take their canoes out; otherwise, the strong surf near the shore during rough weather prevents them from launching their boats. The topography and presence or absence of the mud banks seem to influence the fishing operations here during these early south-west monsoon months. It is possible that during these months also good catches of fishes can be obtained if it were possible to carry out fishing operations.

Similar observations on the pelagic fisheries and plankton production made elsewhere also lend support to those recorded here. In the case of the Atlantic mackerel, *Scomber scombrus*, according to Sette (1950, pp. 294, 305) " mackerel catches coincided with plankton concentrations to suggest that the mackerel tended to travel or tarry in waters richest in plankton content. However, if the feeding of the mackerel reduces a zooplankton concentration rapidly severely, one would expect an intially positive correlation between mackerel and zooplankton to become a negative one as feeding proceeds ". After giving more details about the feeding of the mackerel, Sette (*l.c.*, p. 295) observes that " while there is no evidence that local mackerel and zooplankton concentrations tended to coincide with each other, the agreement of plankton abundance and the presence of the mackerel in general suggests that evolutionary processes have brought about a habit pattern in which this species reaches various areas along its route of spring migration at a time when, on the average, feeding conditions are favourable". It is possible that what has just been stated here about the Atlantic mackerel may prove equally true of the oil-sardine and the mackerel of this coast of India.

The other example concerns the *Calanus*-herring relationship in the northern waters which has been studied in great detail from all aspects—production of *Calanus*, abundance of same, feeding of herring on *Calanus* and so on—by several workers (Bigelow, 1922, 1926; Bogorov, 1934; Boldovsky, 1941; Bowers and Williamson, 1950; Cheng, 1941; Cushing, 1955; Farran, 1928; Fish, 1936; Fuller, 1937; Fuller and Clarke, 1936; Gibbons, 1936; Hardy, 1924, 1926 *c*; Harvey, 1937 *a*; Henderson, 1936; Henderson *et al.*, 1936; Jespersen, 1928, 1932, 1936, 1944; Jørgensen, 1924; Lucas, 1936 *b*; Marshall, Nicolls and Orr, 1939; Marshall and Orr, 1952; Ogilvie, 1934; Rees, 1949; Ruud, 1929; Sanders, 1952; Savage, 1926, 1931, 1937; Wimpenny, 1933, 1936 *a*). These investigations show that the production of *Calanus finmachichus* depends on phytoplankton production,

that the herring feed on *Calanus* most intensively in May and June and at that time *Calanus* is present in great abundance and is available to the herring and that the herring landings are related to the presence of *Calanus*. It has been found that abundance of *Calanus* and good landings of herring coincide and scarcity of *Calanus* portends poor landings of the fish. Savage (1937) and Cushing (1955) have clearly brought out the relationship between herring landings and *Calanus* depletion based on the former's feeding habits. Somewhat similar relationships have been found between *Calanus finmachichus* and sei whales, *Balaneoptera borealis* (Hjort and Ruud, 1929) and also between *Euphausia superba* and the blue and fin whales in the Antarctic (Mackintosh, 1934; Hardy and Gunther, 1935).

On the west coast of India as mentioned already, the patch of phytoplankton consisting of mainly *Fragilaria* during the peak period, on most of the occasions, is very extensive. Hence, it may be presumed that, as observed by Cushing in the case of the herring, the oil-sardine shoals scatter to their minimum size before or during the first stages of feeding on *Fragilaria*; and as long as there is an abundance of *Fragilaria* (as during June and July and sometimes August) searching within the patch will be at a minimum and therefore the shoals will be small; the catches of oil-sardine during this period also is small as in the case of the herring in the Shields area. As *Fragilaria* thins out, searching within the patch, now broken into a series of smaller patches (in the early part of the period of bloom, the content of *Fragilaria* in the water is more or less uniform and occurs on almost all days, but later on it is seen on some days only and on other days is absent, probably a result of the breaking up of the patches due to the feeding effect) will increase, and the shoals, no longer dispersed by continuous feeding activity, might also increase in size. Hence, there might be a relation here also, as in the case of the herring, between the length of time, between complete aggregation and complete annihilation, and the subsequent level of the landings. If this period is prolonged there is time for shoals to thicken up as they ' mop up ' the *Fragilaria*. It is possible that here also, the bulk of the fishery is a feeding fishery. The level of landings, therefore, in such a fishery, is directly related to the time for destruction of the *Fragilaria* patch. It may be mentioned that the landings of the oil-sardine are of small magnitude during the beginning of the season perhaps due to the smaller shoals encountered and also, on this coast, due to limitations of fishing operations caused by inclement weather; and, as in the case of the herring, the landings increase later on when the *Fragilaria* patch is almost ' mopped up ' and larger shoals are encountered. The peak of the fishery is attained then when there is apparently no *Fragilaria* in the water. In the period following, it has been

found (Nair, 1952) that when *Fragilaria* occurred, oil-sardines were also available on some occasions. It would appear that high landings of oil-sardines is related to an early and rich supply of *Fragilaria* as in 1953.

It is an interesting fact that both sardines and mackerel appear earlier in the south and slowly extend northwards and their disappearance follows a reverse pattern (Panikkar, 1952, p. 765). It was mentioned earlier that the south-west monsoon breaks earlier in the south and the bloom of phyto-plankton and zooplankton also begins earlier in the south and spreads north and the current circulation at this time is from the north to south; it is possible that the migration of the fishes at this time is due both to the increasing bloom of plankton, its food, earlier in the south and its spread-ing north and also their tendency to swim against the flow of current. In the latter period, the current becomes reversed and flows from the south to the north and the bloom of plankton during this period depends more or less on the influx of the Bay of Bengal water into the Arabian Sea and, probably, the intensity of the bloom is greater as one proceeds south; and, the move-ment of the fishes and their disappearance starting from the north to the south would appear to be correlated with the bloom of plankton and their tendency to move against current. Therefore, the sequence of appearance and disappearance of these fishes may be related to the water movements and plankton bloom; and, it is possible, as observed by Sette (1950, p. 295) that these fishes reach various areas along their route of migration at times, when, on the average, feeding conditions are favourable.

XVI. Magnitude of Fish Production in Comparison with Phytoplankton Production and the Prospects of Future Harvests from the Sea on the West Coast

In the earlier sections of this acount, the magnitude of the standing crop and the interrelationships between phytoplankton on the one hand and nekton on the other have been dealt with. The high fertility of the waters on the west coast of India and the factors governing the production of phyto-plankton will be discussed in Part II of this paper. As man has to depend on the fishery resources to partake of the production of organic matter in the sea, it would be interesting here to assess the magnitude of the total quantity of phytoplankton produced annually in our waters and how it compares with the landings of commercial fishes. The answer should throw some light on the intensity of exploitation going on at present, and whether there is scope for increased exploitation in the future, or whether we are already depleting the stock. The magnitude of plankton production in relation to fishery resources have been reviewed by Riley (1950) and Merriman (1950)

who also advocate culture of oysters and clams, which are found at the bottom of the food chain, to make the best of this huge production of matter by the minute organisms in the water (*see* also Clarke, 1950).

The problems concerning the fishery resources, conservation, exploitation and research and so on in India have been dealt with by many (Rao and Panikkar, 1949; Rao, 1949; Chopra, 1951; and Panikkar, 1952). It is generally expected that with improved technique and gear as well as exploitation of wider areas, increased landings would result, but, as yet, we have no scientific basis to support this expectation; however, it is interesting to note that recent attempts at fishing with the aid of mechanized vessels off Bombay have yielded increased output (Panikkar, 1952, pp. 748-49). The vagaries of fish landings have led some even to believe that already we are over-exploiting the resources. Therefore, it would be worthwhile to examine, at present somewhat on a hypothetical basis, the extent of the fishery resources as could be calculated from the results of the investigation of the phytoplankton on the west coast described in this paper.

The magnitude of the production of phytoplankton in different waters has been estimated by various methods by several authorities and, generally, it has been found to be several times that of commercial landings of fish. Riley (1950) states that this could be even 1,000 times the commercial catch of fish. Several methods have been adopted in arriving at the estimation of production of phytoplankton and organic matter in the hydrosphere,—oxygen production during photosynthesis, utilization of carbon dioxide and nutrient salts (referred to below; *cf.* also Moore *et al.*, 1914; Gaarder and Gran, 1927; Gran and Thompson, 1929–31; Steemann Nielsen, 1932, 1937 *b*, 1951 *b*; Sverdrup *et al.*, 1942, p. 938) and, recently, radioactive carbon, C^{14}, has been employed in the estimation of the production of organic matter (Steemann Nielsen, 1951 *a*, 1952, 1954). Some of Riley's estimations for the American waters, particularly the Sargasso Sea (Riley, 1939) and of Seiwell (1935 *a*) for the tropical western north Atlantic are considered too high by Steemann Nielsen (*l.c.*, *cf.* however Riley, 1953). Riley (1944) has given an estimation of the production of organic matter for the whole world, water and land (*cf.* also Clarke, 1946; Kalle, 1948; and Krey, 1953).

Some of the estimations of the production of phytoplankton are given below (1–8 from Gilson, 1937) as they are of interest in the discussion that follows:—

Author	Region	Method	Production: grams/metre/square/day wet weight
1. Atkins (1923) ..	English Channel	CO_2	8·3
2. Cooper (1933 *b*) ..	English Channel	Various	8·3
3. Marshall and Orr (1927) ..	Clyde	O_2	26·3
4. Gran (1927) ..	Bergen	O_2	6·7
5. Gran (1927) ..	(Culture)	O_2	25·0
6. Gran (1929 *b*) ..	Ramsdalsfjord	O_2	2·14–9·94
7. Føyn (1929) ..	Lofoten	O_2	18·6
8. Seiwell (1935 *a*) ..	Tropical Atlantic	O_2	12·7
9. Gilson (1937) ..	Arabian Sea	NO_3	14·4

It was not possible here, owing to the limitations of equipment, etc., to estimate production by any of the methods mentioned above. Nevertheless, based on the high nutrient salt content of the water on the west coast,* the high standing crop of plankton noticed in terms of pigment units and dry weight and of zooplankton, etc., and a higher rate of metabolism possible in this tropical environment, it may be assumed that the production of plankton here is very much higher than in some of the temperate regions. The bulk of the producers, the Diatoms, are very much similar in the nature of their composition. The ratio of P: N: Si in the waters of the present region is the same, as in the temperate regions like the English Channel (Part II). The ratio of these elements in the plankton may also be similar as also the mode of intake and assimilation; but, the temperature conditions as well as illumination do not appear to be limiting factors for the maximum production of life in these waters.

Gilson (1937) states that the production in the Arabian Sea calculated by him is of a higher order than in many temperate regions, particularly the English Channel. Gilson's estimation was during a period, as revealed by the present investigation, after the peak of production of phytoplankton had waned. Even then, comparison of the standing crops recorded by him and

* This aspect is discussed in Part II of this account.

in the present area by the writer shows that production is several times higher at the Calicut stations during the same season. Using the C^{14} technique, Steemann Nielsen (1954) found that the production near the mouth of the English Channel to be of the same order of magnitude as estimated by the Plymouth Laboratory from the decline of phosphate values of spring to summer (Atkins, 1923; Cooper, 1933 *b*); considering the period of maximum production of phytoplankton to be from March to June, the value found was 470 milligrams C per square metre per day. In a similar manner, the maximum production recorded by him in the Indian Ocean was 332 milligrams C per square metre per day in a region near the southern end of the Indian Peninsula (Steemann Nielsen, 1952). (The time of the year is not stated.) The above value for the Indian Ocean is stated by the author to be low by 30% and, therefore, the corrected value comes to nearly 432 milligrams, not much different from that found for the Channel during the peak season. The production, when evaluated after considering all the points noted above, for the west coast of India over the continental shelf a wider area of greater depth than that investigated (15 metres), a very highly fertile region, is likely to be several times that found in the English Channel, *at least* 6 *times* on the average.

For the English Channel, Cooper (1933 *b*, p. 744) has calculated the intensity of phytoplankton production based on the utilization of phosphate, nitrate, silicate, carbon dioxide and oxygen similar to the earlier estimations made by Atkins (1922, 1923). The minimum production calculated by him for an area of 82,100 square kilometres and of 72 metres depth on an average, as per rate of production cited above, was found to be 115 million metric tons annually. The total landings of commercial fish in the English Channel ports in U.K. and French coast in the year 1928 was 71,000 metric tons; the fraction of fish landed to total production of phytoplankton works out to 0·0006 or 0·06% only in an area very intensively exploited by modern methods; probably, that is all what can be obtained as fish out of the total production of matter.

In a similar manner, on the basis of the higher rate of production on the west coast of India (6 times) compared with the Channel, the phytoplankton production may be estimated and correlated to commercial landings. The present area fished on the west coast of India extends approximately 1,200 miles by 7 miles with depth not exceeding 40 metres. The potential fishing area could easily be 1,200 miles by 50 miles (nearly half the width of the continental shelf) equalling 60,000 square miles, *i.e.*, 155,400 square kilometres

and to a depth of 100 metres.* Therefore, the production of phytoplankton at the rate mentioned above in an area of 1,55,400 square kilometres amounts to:—

Production in the English Channel = 1,400 metric tons per sq. metre sea surface of depth 72 metres (Cooper, 1933 *b*).

Production of phytoplankton on the west coast of India per sq. metre sea surface of depth 100 metres at 6 times the value of the Channel in 155,400 sq. kilometres

$$= \frac{1{,}400 \times 100 \times 6}{72} \times 1{,}55{,}400$$

$$= 1{,}813{,}000{,}000 \text{ metric tons.}$$

Data available from the statistics of marine fish landings in India show an average catch of 5,61,900 metric tons annually (average of five years landings, 1950–54, based on Central Marine Fisheries Research Station Annual Report, 1955) from the Sea; of this quantity 4,55,900 metric tons, *i.e.*, 80% are landed, on an average, on the west coast. The probability of the west coast being more productive has been indicated by Panikkar (1952, p. 756).

Therefore, the proportion of fish landed on the west coast to phytoplankton production

$$= \frac{4{,}55{,}900}{1{,}813{,}000{,}000} = 0{\cdot}00002515, \textit{ i.e.}, \ 0{\cdot}0025\%.$$

This low percentage would indicate that only a small fraction of fish produced appears to be landed assuming that the same proportion of phytoplankton is ultimately converted into fish as in the Channel.

This aspect of the question may now be examined in the light of the results obtained by the *John Murray Expedition* (Gilson, 1937, p. 59) in the Arabian Sea. The value of phytoplankton production calculated amounted to 14·4 grams per metre-square per day for a depth of 55 metres in the open sea. The value was arrived at based on nitrate consumption during a period of five months from *end of September to end of February*. As pointed out in the present account, the bloom of phytoplankton during this period of the year is far less than that during the earlier period, the south-west monsoon,

* In the North Atlantic, according to Riley (1939) phytoplankton can occur up to 400 metres depth; so the basis of 100 metres cannot be an overestimation.

the period of maximum production on this coast. The production of phytoplankton in the region studied by the *Expedition* also, during the south-west monsoon season, is definitely likely to be greater; hence, the value found by the *Expedition* is a very low estimate and, even as such, as pointed out earlier, is of a higher order. Therefore, the surmise made here that, on an average, the production in the present area is likely to be 6 times that of the Channel, is not far wrong, for, nearer the coast on the continental shelf the fertility of waters is also known to be higher (Sverdrup *et al.*, 1942, p. 944) and, on the west coast of India, it is more so for several reasons already dealt with (*see* also Part II). Further, the record of the standing crop of phytoplankton estimated by the *Expedition* is of a far lesser magnitude than that found on this coast. Nevertheless, a comparison of the magnitude of production of phytoplankton at the same rate observed by the *Expedition* and the fish landings may be made. The production at the rate of 14·4 grams per day per square metre for a depth of 55 metres and extent of the fishing area assessed above of 155,400 square kilometres, works out to 820,000,000 metric tons and the proportion of fish landed, 455,900 metric tons, to production of phytoplankton, works out to 0·00005558 or 0·0056%.

In both instances, it is seen that the quantity of fish landed is of a lower magnitude when compared with an intensively exploited area like the English Channel where the percentage of landings works out to 0·06%, nearly 10–30 times the values arrived at for the present area. It may, therefore, be stated that on the west coast of India, a more intensive exploitation of the sea over a wider area within the continental shelf alone is likely to increase the harvest from the sea considerably. " Quite possibly ", as Riley (1950) observes, " the world's fish catch could be increased five or ten times or more". This may possibly apply to the west coast of India also. As Steemann Nielsen (1952, p. 138) states, " the investigation of the production of matter can give valuable information on local possibilities of large-scale fishing".

XVII. SUMMARY

1. A detailed quantitative and qualitative study of the phytoplankton, the main groups comprising the same and the total zooplankton, extending over five years at a station on the south-west coast of India, at Calicut, is reported and the interrelationship between plankton production and fish landings discussed. This is the first time such a detailed investigation lasting over several years has been attempted in a tropical environment and in India in particular.

2. The physical and climatic features of the area is given including particulars relating to ocean currents and sea bottom. It is shown that

Calicut is very much typical of a large extent of the west coast of India and is essentially a tropical environment.

3. The biological year here may be said to commence in the middle of April when the first signs of the oncoming south-west monsoon become evident.

4. Standard methods have been employed in the study of the plankton. Plankton from net samples, particularly vertical hauls, and water samples, have been studied by enumeration, extraction of plant pigments and estimation of dry weight, for making a quantitative and qualitative assessment of the standing crop.

5. The fluctuation of the standing crop in terms of the number of phytoplankton organisms and plant pigment units (Harvey Units) is almost identical. The standing crop of phytoplankton is at its maximum during the south-west monsoon months (May to September–October), attaining the peak in July; from then on falls reaching the minimum in November. There are one or more pulses of production during the north-east monsoon season also (October–November to April).

6. The total quantity of phytoplankton shows variations from year to year depending on the nature of the flora concerned in the blooms.

7. The standing crop during the south-west monsoon is predominantly composed of phytoplankton and during the north-east monsoon season, zooplankton constitutes the bulk of the crop.

8. In terms of dry weight, the fluctuation of the quantity of plankton is not marked by such large oscillations as seen in the former instances. A striking point noticed was that of a close approximation between the ratio of the standing crop during the south-west monsoon season (*bulk* of phytoplankton) and the north-east monsoon season (*bulk* of zooplankton). The ratio was found to be 1 : 0·9, and it is surmised that the quantity of living matter that a mass of water can support, the *biomass*, is in equilibrium irrespective of the nature of the constituents, plant or animal.

9. The magnitude of the standing crop in terms of the number of organisms, plant pigment units and quantity of carbon, in a unit volume of water, is shown and compared with similar observations from several regions in the tropical, temperate and Arctic waters. It is seen that the standing crop in the present area is of a very high order and the area is one of the most fertile regions of the world for phytoplankton production.

10. Some particulars are given relating to the horizontal and vertical distribution of the phyto- and zoo-plankton.

11. The phytoplankton is composed of mainly by the Bacillariophyceæ (Diatoms); the Dinophyceæ, Cyanophyceæ, Silicoflagellatæ and Coccolithineæ occur next in the order mentioned. The Bacillariophyceæ constitute the *bulk* of phytoplankton throughtout except on rare occasions when the phenomenon of " discoloured water " occurs owing to the dominance of a single organism in the water to the total exclusion of others.

12. The seasonal fluctuation of the Bacillariophyceæ follow closely that of total phytoplankton outlined above as they form the bulk of the flora.

13. The Dinophyceæ also have a peak during the south-west monsoon season, in June or July, and one or more peaks of production during the north-east monsoon season.

14. The Cyanophyceæ (Myxophyceæ) attain the maximum development during the north-east monsoon season in the warmer months. The other groups occur sporadically during the year.

15. The total number of zooplankters increases with the increase of the Diatoms. The zooplankton composed of smaller forms shows a peak in August, during the south-west monsoon season, immediately after the peak of the phytoplankton. During the next season there are one or two pulses of development composed mainly of larger forms.

16. The phenomena of " discoloured water " observed are described briefly.

17. The seasonal fluctuation of phytoplankton and zooplankton is compared with the earlier observations in India and elsewhere and discussed.

18. The phytoplankton-zooplankton relationship observed here is described and discussed with data from other localities.

19. The relationship between commercial fish landings, and phyto- and zooplankton standing crop is indicated. A close relationship was found between the standing crop of phyto- and zoo-plankton with the total quantity of fish landed, particularly with the landings of the oil-sardine and the Indian mackerel, which are plankton feeders. An intimate relationship appears to exist between the oil-sardine and the Diatom *Fragilaria oceanica*, which occurs in large quantities during the south-west monsoon season, and this relationship is discussed.

20. The magnitude of the phytoplankton production on the west coast of India has been tentatively estimated and contrasted with the total landings of commercial fish on the west coast. It is found that the fish landed represents only a very small fraction of the total production of phytoplankton, and compared with an intensively exploited area like the English Channel, it is pointed out that there is vast scope for increased exploitation with positive results on the west coast of India.

(Literature and Acknowledgement at the end of Part II)

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STUDIES ON THE PHYTOPLANKTON OF THE WEST COAST OF INDIA

Part II. Physical and Chemical Factors Influencing the Production of Phytoplankton, with Remarks on the Cycle of Nutrients and on the Relationship of the Phosphate-Content to Fish Landings*

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Received March 13, 1958

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I. INTRODUCTION

IN the first part of this series of studies, the biological investigations on phytoplankton and zooplankton crop, their interrelationships, the magnitude of the standing crop, an assessment of total phytoplankton production and its relationship to fish landings, etc., were reported. In the present account, the environmental factors, physical (such as temperature of the water, light intensity, ocean currents, wind force, etc.) as well as chemical (such as salinity of the water, nutrient salts—phosphates, nitrates, silicates—dissolved oxygen, etc.) are described and their bearing on the fertility of the waters, phytoplankton production, and to fish landings discussed.

The methods employed in the analyses of sea-water are the same as those described by Harvey (1928 *b*, 1945; *see* also Jayaraman, 1951, 1954). A hydrological register is being maintained at the Central Marine Fisheries Research Substation since October 1949, by the collaboration of the several members of the scientific personnel including the writer and the chemical data presented here are drawn from this register. During the period June 1955 to November 1956, the writer was in charge of all aspects of hydrological studies. The interpretations are the writer's own.

II. TEMPERATURE OF THE WATER AND LIGHT INTENSITY

(*a*) *Temperature*

The sea surface temperature data recorded relate to the stations from where plankton samples were actually collected, 5 to 6 miles from the shore. At Calicut, the sea surface temperature (Text-Figs. 1 and 7) is lowest in July being 25·3° C. and highest in April being 30° C. (average of 5 years). There is a tendency for the water temperature to fall slightly (a degree or so) in January from the value obtaining in December, after which it rises again.

The sea surface temperature also presents a double oscillation during the year as does the air temperature. The main maxima occur in April (1950, 1952–54) or May (1951) and the secondary one in October (1953), November (1949, 1950, 1951 and 1954) or December (1952) (Text-Fig. 1). The minimum values occur in July (1950), August (1951 and 1952) or Septem-

ber (1953 and 1954). A similar bimodal or double oscillation of the sea surface temperature has been found to be common for several of the warmer areas investigated in the regions around India (Weel, 1923; Sewell, 1929; Bal *et al.*, 1946; Chevy *et* Serene, 1948; Chidambaram, 1950; Kow, 1953; Chacko, 1956; Chacko *et al.*, 1954; *see* also Prasad, 1957). Observations made by Ganapati and Murthy (1954) in the Bay of Bengal off the Vizagapatnam coast show a triple oscillation of the surface temperature with a difference of 4·4° C. between maximum and minimum. Lowest values occurred in January and highest in October as at Madras. Ramamurthy (1953 *b*), however, could find no double oscillation in the temperature of the water off the Madras coast; his values vary from 27·16° C. to 31·1° C. in January and October respectively.

It may be seen from the above that the nature of fluctuation in the surface temperature of the water is not very different over a wide area in and around Indian waters in the tropics.

It was not possible to record the temperature differences in relation to the depth of the water; a few observations made in the beginning of the investigation showed that even at a little depth of about 15 metres, the bottom values were slightly lower. Prasad (1952) and La Fond (1954) have made some observations in this regard in the Bay of Bengal while on short cruises.

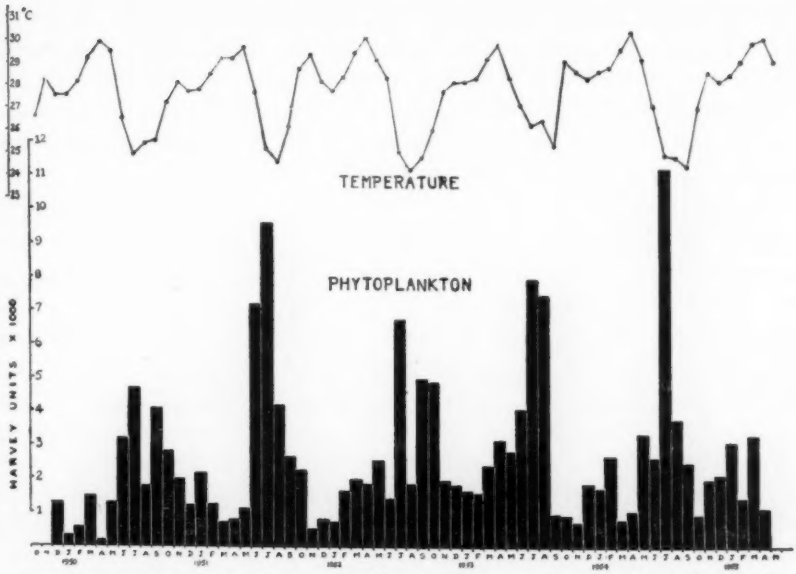
Sewell (1929, p. 212) who studied the Laccadive Sea has also recorded a double seasonal oscillation of the surface temperature as well as air temperature. The other points made by him are: In the Indian waters the surface temperature is normally higher than the air temperature at all times of the day; the sea temperature when it becomes lower than the air temperature does so at or about midnight, and as the temperature of the air normally falls more rapidly than that of the sea-water, it would further seem probable that at this time of the night there is some phenomenon occurring which causes the abnormal lowering of the surface temperature; the alteration in the relationship of the two temperatures ultimately depends on the strength of the wind; the wind brings about a diurnal variation in the temperature, a rise in wind force being accompanied by a fall in the temperature; during the winter months, the temperature of the sea is colder at the surface than at a depth of 20–50 metres, particularly at night or early morning hours; increase in the wind force will tend to cool the surface, but increase the amplitude of the waves and bring about admixture of surface and bottom waters thus altering the temperature relationship of the air and the sea surface and resulting in the temperature difference; during the hot dry months of October, April and May, increase of wind force causes lowering of surface

temperature and gives rise to an admixture of surface water with water from some depth which is at a lower temperature, both facts thus decreasing the temperature difference between sea and air so that in these months the wind force and temperature difference vary in opposite directions; there is thus a double diurnal upwelling of water from some depth below towards or actually on to the surface. Obviously, in the present area, such diurnal changes must be constantly replenishing the surface layers with nutrients which also accounts for the high production of plankton seen here.

It may be mentioned that during these months concerned, October, April and May, but for the increase in the wind force (Text-Fig. 8), the surface temperature would still rise very much.

According to Carpenter (1887 in Sewell, 1925, pp. 47–48, Chart VI), the Cold Antarctic Flow, which makes up for the greater evaporation, is probably uniform in temperature and widely spread; it extends up to 10° N. and gradually surfaces. One arm of this current extends to the Arabian Sea. This current may have considerable influence on the temperature conditions also in the Arabian Sea besides on the salinity and nutrient salt content. Further, the waters of the Antarctic Intermediate Current which rise towards the surface from great depths as they move north along the East African Coast (Mohamed, 1940, p. 193) also enter into the current circulation of the Arabian Sea and thus the west coast of India and affect the chemical and physical properties of the water including the temperature.

An examination of Text-Fig. 1 shows that the main bloom of phytoplankton occurs when the water temperature is falling, though the lowest temperature attained is only about 24–25° C. But phytoplankton bloom also occurs even at higher temperatures of about 27–28° C. Generally, when the water temperature goes above 29° C., the phytoplankton content is either poor or unhealthy and the elements are found dead and degenerating. Marshall (1933, p. 104), however, states that a rise in temperature had no depressing effect on the Diatom population in the Great Barrier Reef. It is known that a linear relationship exists (between 15 and 25° C.) between the temperature and growth below the former's optimum value and this optimum varies with the species (Barker, 1935). Gran and Braarud (1935) found that in the Gulf of Maine, different species of Diatoms showed maximum growth at different temperatures which varied from 3–12° C.; even species within the same genus show different temperature preference. Spencer's (1954, p. 278) experiments showed that the mean generation time falls with increasing temperatures reaching a minimum at about 20° C.; further increase had no effect on growth though there was no inhibiting effect



TEXT-FIG. 1. Seasonal fluctuation of the temperature of sea-water (surface) and phytoplankton crop (Harvey Units). Explanation in text.

up to 25° C.; above 25° C., adverse effects came to be noticed. According to McCombie (1953), in freshwaters, the seasonal cycle is caused chiefly by the seasonal change in the water temperature resulting from the change in the solar radiation. Wallace (1955) states that the optimum and maximum temperature tolerated respectively by some freshwater Diatoms are 26–20° and 34° C. for *Nitzschia filiformis*, 22 and 30° C. for *Gomphonema parvulum*.

The range of temperature here in this tropical environment is not much. It has also been shown above that there is a constant mixing up of the layers which tends to bring an equilibrium in the temperature conditions and during the south-west monsoon season this is very pronounced owing to upwelling of the deeper waters. It is interesting to note that the peak of phytoplankton production occurs on the west coast of India when the water temperature is between 24° and 25° C. resembling the optimum temperature conditions noticed in the cultures by several workers cited above. Temperature here, most of the time, appears to be at about optimum levels for production of phytoplankton.

(*b*) *Light*

In a region such as the present one with the average hours of sunshine exceeding nine hours a day, light cannot, obviously, be a limiting factor for

growth of phytoplankton, as it is so in the higher latitudes, the temperate and polar seas. The stations sampled are situated within an area where the depth does not exceed 40 metres. It is well known that photosynthesis can take place even at 100 metres depth (Clarke, 1936) though often this *compensation point* may vary according to the transparency of the water which depends on the amount of suspended matter present in it.

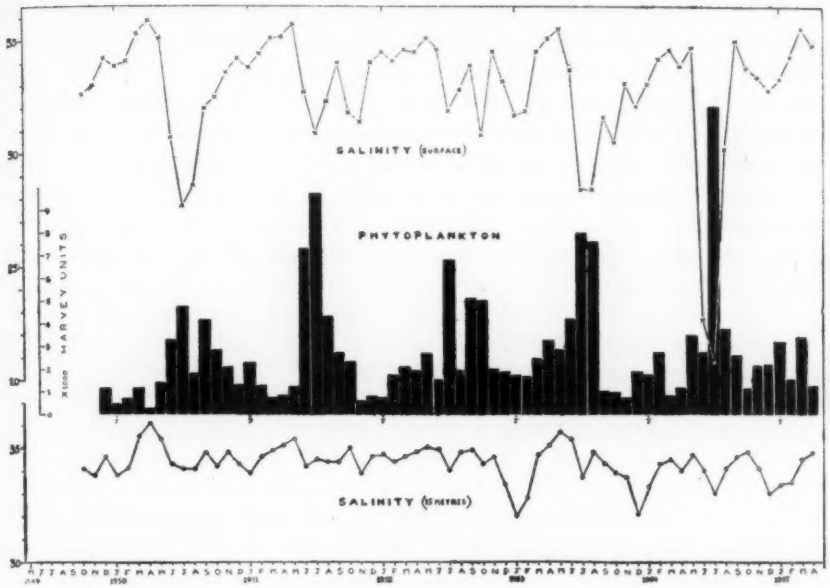
The influence of light on the growth of phytoplankton has been studied in detail in the higher latitudes (Stanbury, 1931; Gaarder and Gran, 1927; Gran and Braarud, 1935; Marshall and Orr, 1928; Jenkin, 1937; Petterson, 1936; Petterson, Höglund and Landberg, 1934) as also the quality of light utilized (Clarke and Oster, 1934; Oster and Clarke, 1935; Clarke and James, 1939; *see also* Harvey, 1955). Chidambaram and Menon (1945, p. 365) state that " lack of sunshine " and " unstable conditions " arrest the development of plankton organisms during the south-west monsoon season on the west coast of India. These authors have no data for the months of June and July, the peak of the monsoon period as no collections could be made then by them. The investigations covering a period of nearly 6 years by the writer shows that it is *during these very months* that the phytoplankton attains its zenith of development on the west coast of India.

III. SALINITY OF THE SEA-WATER

The values recorded for salinity of the sea-water are shown in Text-Fig. 2 on the basis of monthly averages. The salinity of the surface water fluctuates more sharply than that of water at a depth of 15 metres. At the surface, the maximum values occur in April or May and the lowest in July, during the south-west monsoon. The salinity rises after July and there is a second fall in October, November or December from when on there is a rise till the drop in the following year with the beginning of the south-west monsoon.

The variation in salinity in parts per *mille* was as follows: at the surface, 27·68–35·14; 30·93–35·65; 30·80–35·05; 28·35–35·47 and 20·74–35·54; at the bottom, 33·89–35·43; 33·93–35·43; 32·76–35·11; 32·08–35·72 and 33·02–34·69 during the years (May–April) 1950–51, 1951–52, 1952–53, 1953–54 and 1954–55 respectively.

Generally, salinity at 15 metres depth is higher than that at the surface but on some occasions (January and February 1950; March, April and May 1951; March 1952; and March, May and September 1954) the salinity of the bottom samples was slightly less than the surface value. This is very possibly due to the evaporation of the surface water on account of the higher



TEXT-FIG. 2. Seasonal fluctuation in the salinity of the surface and bottom water, and of phytoplankton (Harvey Units). Explanation in text.

temperature prevailing at those times, or incursion of low salinity water from below through currents.

The greatest variation between surface and bottom values occurs during June–July of the south-west monsoon period and the least during the north-east monsoon period, November to April or May.

It may be seen that there is more or less a triple oscillation in the fluctuation of the salinity during a year in these waters, two of which are clearly marked.

At 15 metres depth, the salinity changes are more gradual and the lowest average value recorded is 32‰ (January and December 1953) unlike the surface layer where the lowest value occurs in June–July. While the surface salinity shows a very appreciable fall, sometimes to even as low as 21‰, during June–July, the height of the south-west monsoon, the bottom values are as high as 33–34‰ at the same period. The rainfall at sea beyond 20 miles from the Malabar coast is very much less (Subrahmanyan, Part I) hence salinity would appear to attain an equilibrium soon and the overall fall in salinity during the period of maximum rainfall does not exceed 3% of the high values obtaining before the onset of the south-west monsoon.

The higher values for salinity are recorded during periods when the air and water temperatures are high, wind force greater and humidity low, factors which permit evaporation. It is during such periods that the range of salinity between the surface and bottom layers is least, probably because of the higher wind force obtaining at the time which leads to the mixing up of the water layers. Sometimes the surface values for salinity exceed those of the bottom slightly, as indicated already, due to the heating up of the water and consequent evaporation and lack of mixing up of the layers by wind. Very often, during the summer, when wind force also is high, the surface and bottom values are the same.

The lower values for surface salinity occur with the fall of temperature, occurrence of rainfall, increased humidity and fall in the strength of the wind force which does not allow of the mixing up of the water layers. During this time, the difference in the salinity of the surface and the bottom is greater.

Like temperature, salinity also shows a well emphasized double oscillation and a third minor one. When dealing with the temperature changes of sea-water, it was mentioned that, according to Sewell (1929) there is a diurnal change of temperature depending on wind force and this leads to an upwelling of the water from the lower layers towards the surface bringing about changes in the temperature of the water. Sewell (1929, pp. 274–75) has shown that salinity also is affected by the same forces. According to him, in the Indian seas, there is a distinct double oscillation during the course of the day in the salinity of the surface waters; this is correlated with the double oscillation of the wind force during the twenty-four hours of the day. This double oscillation of the salinity exhibits two phases at different periods of the year; at one period, salinity rises and falls with the variation in the strength of the wind, while at another period, it oscillates against the wind force, the oscillation and phases depending on the relative salinity of the surface water and that at the bottom which wells upwards under the influence of the wind. During the wet monsoon months, the surface water becomes diluted and rendered less saline either directly by rainfall or indirectly by the increased outflow of river-water and the oscillation of the salinity is the positive phase, *i.e.*, an increase in the wind force tends to increase the salinity of surface water by bringing up water of higher salinity from below; whereas during the hot dry months, the surface water is more saline due to evaporation and an increase of the wind force brings about a diminution in the salinity owing to the mixing up of lower salinity bottom water with the highly saline surface water and this phase is the negative one.

Sewell's observations cover only the months, October to May. According to him, in the Laccadive Sea, the oscillation phase is positive in October and November indicating that the surface value of salinity is less than that at some depth owing to the effect of the south-west monsoon. In December, the phase is negative and remains so in February and March; in April and May, it is either positive or negative depending on local condition. In the present area in the Laccadive Sea, investigated by the writer, which has been covered throughout all the months for over 5 years, the oscillation phase is positive throughout the south-west monsoon period of May to September and up to December generally, the only exception being September 1954 (when the bottom values were lower than the surface values slightly); from January to May, the phase may be positive or negative.

The north-east monsoon, according to Sewell (1929, p. 338) seems to have no effect on the relationship of salinity of the surface water to that in the deeper layers. The effect of the north-east monsoon is felt more on the eastern side of the Peninsula than on the western side as evidenced by the range of temperature, increase of humidity, etc.

The oscillation of the salinity in the present area may be positive or negative from January onwards up to May. The change in the phase from positive to negative mentioned by Sewell (1929, p. 338) for the Laccadive Sea is not felt in the present area, perhaps due to the proximity of the station investigated to the shore. However, one point appears to need emphasis as far as salinity is concerned, *viz.*, that the north-east monsoon active on the east coast of the Peninsula appears to affect the salinity of the water on the west coast; for, the salinity values for both the surface and the bottom water record a fall in December or January, though there is no precipitation on the west coast at this time. This fall in the salinity appears to be due to the coastal current around the Peninsula at this time, which flows in a north-westerly direction, bringing in water of low salinity from the east coast and other areas where the north-east monsoon is active and heavy rainfall occurs.

George (1953 *a*) has tabulated the salinity values for three years, 1948–49 to 1950–51 for the area covered by this investigation including a part of the period and the values recorded are identical; but, beyond pointing out the effect of the heavy rainfall during south-west monsoon on the lowering of the salinity values, other aspects of the question have not been dealt with by him.

The salinity oscillations on the east coast of the Peninsula show some differences. At Madras (Jayaraman, 1951) salinity remains more or less

constant, around 34·5‰ from about March to September, the amplitude of the oscillations being too small. The highest values are attained in April or May as at Calicut. From October to December, low values are recorded down to nearly 24‰ during periods of rainfall. The very low salinity recorded is not only due to the local rainfall at that time, but also to the effect of influx of freshwater discharge into the Bay of Bengal by the large river systems of India which are in floods owing to the south-west monsoon precipitation in the mountain ranges, their source. This flood water enters the coastal circulation thus lowering the salinity of the sea-water (Sewell, 1929, Text-Figs. 78 and 79). Ramamurthy's (1953 *b*) results for Madras are similar to those of Jayaraman; they vary between 23·23 to 34·94‰.

The salinity values recorded by Jayaraman (1954) for three years in the Gulf of Mannar and two years in the Palk Bay show that, high values occur during May to October, south-west monsoon season, and the lower values between November and April, the north-east monsoon season. In the Gulf of Mannar, the values range between 28·85 and 36·47‰, the lower value occurring in January and the higher in September; in the Palk Bay also, the lower values obtain in January and the highest in October, the values varying between 25·52 and 36·39‰ respectively.

Some data relating to salinity estimations in Indian waters is given in Table I for comparison. It may be seen that the variation in values at Calicut is least compared with the other areas which is indicative of oceanic conditions.

Since the main peak of production of phytoplankton (Subrahmanyan, Part I) occurs during or immediately following a drop in salinity values, owing to the monsoon precipitation, the bearing of salinity on the biological content of the water, particularly as it affects the phytoplankton may be examined now.

Salinity, as is well known, is important in maintaining the proper osmotic relationship of an organism. Species of phytoplankton have their own range of toleration to salinity changes (Braarud, 1951). However, not much is known about the osmotic relationship of Diatoms, the main constituents of the phytoplankton; but, Gross (1940) found that the Diatom *Ditylum Brightwelli* does not fit into the normal concept of an osmotic system and the plasmatic membrane of *Ditylum* cannot be regarded as a semi-permeable membrane as transfer of water between the cell and the medium is to a large extent independent of the differences in the osmotic pressure between cell content and the medium. Changes in the volume of *Ditylum*

TABLE I

Salinity in parts per mille in the Indian Waters

Region	Minimum	Maximum	Remarks	References
1. Madras ..	24·00	34·50	Lower value effect of North-East Monsoon	Jayaraman, 1951
2. Madras ..	23·23	34·94	do.	Ramamurthy, 1953*b*
3. Gulf of Mannar ..	28·85	36·47	do.	Jayaraman, 1954
4. Palk Bay ..	25·52	36·39	do.	Jayaraman, 1954
5. Vizagapatnam ..	25·00	34·00	Lower value effect of both monsoons mainly South-West Monsoon	Ganapati and Murthy, 1954
6. Bombay Harbour	23·56	38·40	Lower value effect of South-West Monsoon	Bal *et al.*, 1946
7. Calicut ..	31·00	35·00	do.	Present investigation—Figures average (of 5 years' data) for water column

protoplasts cannot be brought about by changes in the concentration and composition of the surrounding medium and these changes are not governed by the laws of osmotic equilibrium. It would appear that the Diatom cell has a peculiar reaction to changes in the salinity depending on its own physiological state.

One reaction of the Diatoms to changes in the salinity has great significance to the life-history of the organism, *viz.*, a fall in the salinity under certain conditions. A few instances from experiments will be found useful to clarify this point.

Iyengar and Subrahmanyan (1944, pp. 132–33) and Subrahmanyan (1947, p. 245) found in the cultures of Diatoms that the Diatom cells show a period of rapid multiplication by vegetative division and frequent transference of them to fresh culture media helps to keep up the multiplication of the Diatoms concerned; but, there comes a stage when the Diatom cells do not respond to further changes of culture media and no increase by division could be noticed. The cells by this time had considerably diminished in size owing to repeated divisions, a characteristic feature of the Diatoms. If left

in the medium now for long, they begin to degenerate and die. But, if at this stage they are transferred to sterilized water slightly diluted (10–20%) with distilled water, the cells continue to live healthily but do not divide further. However, after some time, they begin to show auxospore-formation, which is a mode of rejuvenation and the process is often sexual; after this, the zygotes germinate and give rise to large cells which in their turn divide vegetatively and continue the cycle. The rate of division in the early stages is very rapid. The authors also found that dilution beyond 20% tends to kill the Diatoms. It has also been found that cells forming auxospores were within a certain range of size, characteristic for each species; cells either larger or smaller do not form auxospores, but ultimately they died in the medium. Somewhat similar observations have also been made by several workers on marine and freshwater Diatoms (*see* Iyengar and Subrahmanyan, 1944, for literature on this aspect; Subrahmanyan, 1947; Wimpenny, 1956; and Harvey, 1945, pp. 129–30). Such occurrences are considered possible by Lucas and Stubbings also (1948, p. 164).

It may be interesting to recall here that according to Gran (1929 *b*) the melting of snows and the inflow of freshwater from land (comparable to rainfall on the west coast of India) is an essential factor for the initiation of the vernal bloom of phytoplankton in the Norwegian waters. For the Gulf of Maine, Bigelow (1926) has made the same suggestion. Gaarder and Gran (1927) found in the Oslo Fjord that abundant phytoplankton is restricted to a surface layer with a relatively low salinity. Between Yorkshire coast and Dogger Bank, the peaks of plankton production (as seen from the Text-Fig. 1 in Wimpenny, 1944) coincide with periods of lower salinities.

Coming to the conditions in the sea off the west coast of India, it is found that during the south-west monsoon months, owing to the abundant rainfall, there is a fall in the salinity of the water which is very considerable at times in the surface layers. Such a steep fall is not noticed in the bottom layers of the water and, moreover, an equilibrium is attained soon, bringing the overall average value to 30‰, *i.e.*, a lowering of 12–15% from the high values obtaining (34–35‰) during the previous months. It is believed (Admiralty, 1950) that beyond 20 miles from the shore, over the sea, the rainfall is considerably less; hence, restoration of an equilibrium in the salinity of the water is facilitated. Further, it is possible that there is a reflux of offshore water following the influx into the sea of river-water as pointed out by Huntsman (1955) which also helps in raising the abnormally low salinity of the surface waters. On occasions when salinity is very low (*circa* 25‰) it is found that the plankton in the water is no longer healthy.

During this period of lower salinity, cells of those species which have attained their minimum characteristic size form auxospores as conditions are favourable. Auxospores of several species as also large cells of many others indicating the occurrence of such a process in the recent past, have been met with in the plankton collections at this period, which confirms the view that conditions are very favourable for this process of rejuvenation of the protoplast of the Diatom cells. Further, there is a plentiful supply of nutrients in the water during this period, phosphates, nitrates, silicates, etc., (refer later below) so that the sea-water at this time of the year, so to say, is an ideal culture medium from which phytoplankton draws its requirements and rapidly multiplies leading to the main bloom of the same. It should be mentioned that not all species react in this manner. That each species may have its own optimum conditions of salinity is shown by the recent study of Braarud (1951) on a number of phytoplankton species.

During the north-east monsoon season also, though the west coast of India has practically no rainfall, salinity values register a fall owing to the water of low salinity from the Bay of Bengal entering the Arabian Sea. Then also, there is a bloom of phytoplankton, a smaller secondary peak, in which the species concerned are different.

The reaction of different species to variation in the salinity values may probably determine the floral composition of the plankton which is never alike for a long time and the species responsible for the peak blooms also change from season to season and from year to year.

Further, it is possible that salinity changes may determine the nature of reproduction of a species to some extent. It has been found that, sometimes, a bloom of phytoplankton occurs even when the salinity is as high as 34 or 35‰. This does not, however, appear to be a result of sexual reproduction and auxospore-formation of the Diatom elements as it appears to be the case during instances when the salinity values are lower. Here it appears to be due to the vegetative multiplication, chiefly of the Diatoms. Such blooms occur during or after a period of strong winds which appear to mix up the water layers and make available certain growth-promoting substances from the lower layers or the bottom sediment; for, there is always a good quantity of the well-known inorganic nutrients like phosphates, nitrates and silicates, present in the water (refer later below), such a change leading to the multiplication resembling renewal of media in the cultures referred to earlier.

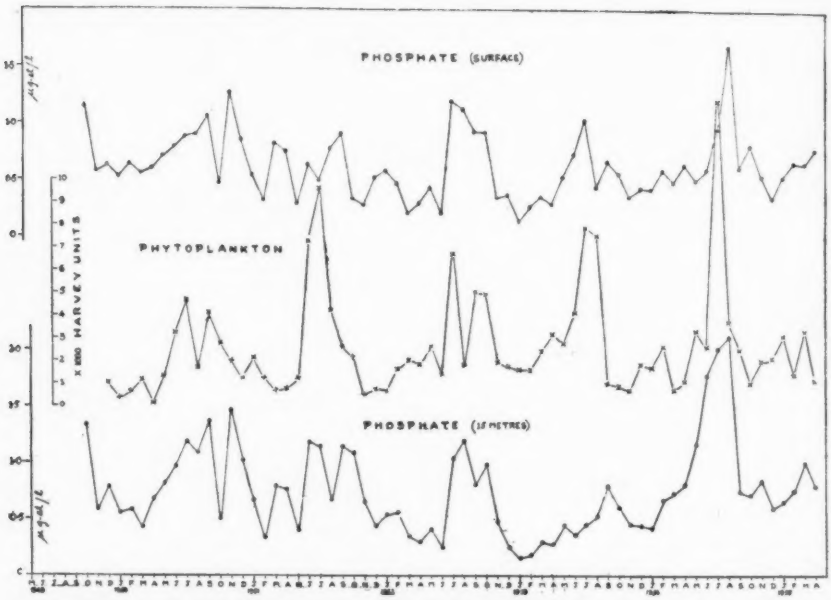
IV. Nutrient Salts and Their Cycle

It is well known now that sea-water is an aqueous solution containing a variety of dissolved salts and gases. Some are present in high concentrations

while others only in very minute quantities. Of these, from the biological aspect, the most important are phosphates, nitrates and silicates; these are the most essential nutrients for plankton growth in the sea.

(*a*) *Phosphate-Phosphorus**

The fluctuation in the phosphate content of the water is shown in Text-Fig. 3 on the basis of monthly averages. The trend of fluctuation at the surface and at 15 metres depth is more or less similar, except that the bottom



TEXT-FIG. 3. Seasonal fluctuation in the phosphate content of the water, surface and bottom, and of phytoplankton (Harvey Units). Explanation in text.

values tend to be somewhat higher generally; but on some occasions, the latter fall below those at the surface, *e.g.*, February–March, 1950; March and December 1951; June, July, September and December 1959; February, March, May, June, July 1953; and October, 1954. Both at the surface and bottom, the lowest values occur in December or January, February or March, and the highest values between June and November. The values fluctuated between 0·46–1·26, 0·31–0·85, 0·20–1·19, 0·13–1·02 and 0·41–1·68 µg. at/L. respectively during the years 1950–54 at the surface; and

* Values not corrected for salt error.

at the bottom, during the same period, 0·42–1·46, 0·34–1·18, 0·30–1·19, 0·16–0·79 and 0·42–2·11 μg. at/L. respectively.

At the surface, the lowest value recorded in any one year was 0·13 μg. at./L. in January 1953, and the highest was 1·68 μg. at./L. in August 1954; similarly, at the bottom, the lowest value of 0·16 μg. at./L. occurred in January 1953 and the highest of 2·11 μg. at./L. in August 1954.

It is seen from the data recorded that there is a well-defined oscillation in the concentration of phosphate in the sea here; the peak occurs generally in the latter half of the south-west monsoon and the minima occur in the north-east monsoon months. There is no complete depletion of this nutrient in the water at any time during the year.

In view of the generally held opinion that the warmer waters are not so productive as the colder seas, the concentration of phosphate in some of the regions where this has been estimated are shown in Table II for comparison, in order that a reliable understanding of the relative fertility of the waters may be obtained.*

The observations at Calicut, reported in this account, show that the values recorded here are fairly high; the lowest monthly average value recorded during the 5 years was 0·13 and the highest 2·11 μg.at./L. The variation of the phosphate content in the *water column* as seen by the average of five years' data is 0·474–1·044 μg. at./L. (Text-Fig. 7). Further, it may be seen that there is a seasonal fluctuation in the phosphate content; from a minimum in January, the value increases gradually and reaches the maximum in July; it remains high till September and thereafter falls. This fact does not appear to have been recognised by the earlier workers on the west coast. In the temperate regions, the oscillation of the phosphate content is a double one; there is a pronounced winter maximum and a subsidiary one in summer. The values oscillate much, sometimes over a wide amplitude when short periods are reckoned with; but, when considered over several years, the mode of fluctuation in the present area distinctly stands out as described above.

In the temperate and polar waters, generally an inverse relationship is found between the phosphate and phytoplankton content of the water; a fall in the former being followed by a bloom of the latter (*vide* Papers of Atkins, Harvey, Cooper, Gran, Braarud, Phifer and Thompson, Riley, Hart and so on under references). Here such an overall inverse relationship is

* The writer has converted to μg. at/L. all values expressed in other terms in the sources quoted in this paper.

TABLE II

	Region	Depth in metres	Conc. PO_4—P in μg. at./L		Reference and remarks
			Lowest	Highest	
1.	Indian Ocean ..	700–1000		3	Sverdrup *et al.*, 1942 (value assessed from figure)
2.	Red Sea ..		0·142	1·126	Thompson, 1939
3.	Java Sea ..		0·03515	0·141	Delsman, 1939
4.	Singapore waters		0·1128	1·28	Kow, 1953
5.	Bombay Harbour		0·1837	0·5324	Bal *et al.*, 1946
6.	Bay of Bengal ..		about 0·2	1·50	Jayaraman, 1951. Once 3·0
7.	Bay of Bengal ..		0·3384	1·198	Ramamoorthy, 1953 *b*
8.	Gulf of Mannar ..		0·15	0·30	Jayaraman, 1954
9.	Palk Bay ..		0·12	0·25	Jayaraman, 1954
10.	Calicut—West Coast of India	Surface 15	0·13 0·30	1·68 2·11	Present investigation
11.	Atlantic Ocean ..	Intermediate layer—1000		2·00	Sverdrup *et al.*, 1942
12.	Caribbean Sea ..	Surface Depths	0·1	2·00	Rakestraw and Smith, 1937
13.	English Channel and Mill Port		0	1·05	Atkins, 1923; 1925, 1926 *b*, 1928, 1930, 1953; Armstrong, 1954, 1955; Cooper, 1933 *a*, 1933 *c*; Harvey, 1928 *b*, 1934 *b*, 1945, 1955; Harvey *et al.*, 1935; Marshall and Orr, 1927
14.	Gulf of Maine ..		0·6	1·61	Redfield, Smith and Ketchum, 1937
15.	Gulf of Maine ..		0·0	0·84	Gran and Braarud, 1935
16.	Gulf of Maine ..			1·5	Bigelow *et al.*, 1940
17.	Block Island Sound	0–30	0·43	2·29	Riley, 1952
18.	Mediterranean ..	Surface 90	0·00282 0·0141	0·04 0·0451	Ercegović, 1934
19.	Pacific Ocean ..			3·5	Sverdrup *et al.*, 1942
20.	Equatorial Pacific		0·0423	1·39	Graham, 1941 *a*
21.	Transequatorial waters 9° N to 7° S at 140° W		0·8	2·4	Austin, 1954; Stroup, 1954; Cromwell and Austin, 1954
22.	Hawaiian waters ..	1000 100		3·0 1·0	McGary, 1955; Seckel, 1955
23.	Eastern Tropical Pacific		0·29	0·81	Wooster and Jennings, 1955
24.	Friday Harbour ..		1·20	2·4	Phifer and Thompson, 1937
25.	Great Barrier Reef			0·161	Orr, 1933
26.	New South Wales		0·2115	0·282	Dakin and Colefax, 1935 *a*, 1935 *b* and 1940
27.	Sub-Tropical Zone of Southern Ocean		0·0705	0·432	Clowes, 1938
28.	Antarctic waters			2·256	Ruud, 1930, Clowes, 1938

not obvious though on a few occasions an inverse relationship was seen on a sample-to-sample basis (*see* also George, 1953 *a*; Prasad, 1956). As in this warmer environment there are no sharp climatic distinctions to retard production, development of phytoplankton is possible throughout the year though with differing intensities, and a fairly high standing crop is present in the water always. The relationship, therefore, between phosphate content and phytoplankton is somewhat different from that generally known and a knowledge of the source of this phosphate appears to be necessary for understanding their relationship and the cycle of phosphorus in the sea on the west coast of India.

Various suggestions have been put forward as to the source of phosphate in the sea. Pearsall (1923) suggested that the heavy rainfall of spring and autumn increased the amount of nutritive salts brought down to the sea, indicating that there is considerable contribution of these salts from the land to the sea. Gran (1931) states that the quantities of phosphate and nitrate which are washed out every year from the land are quite insignificant compared with the great regeneration in the depths of the sea. In the Great Barrier Reef region, there is no evidence of enrichment of the sea with phosphate by river outflow though the latter was rich in silicate (Orr and Moorhouse, 1933). According to Riley (1937–38) the Mississippi river brings in large quantities of phosphate into the Gulf of Mexico and the concentration of this salt in the latter is nearly four times that found in the surrounding region. In the south-western area of the English Channel, Hickling (1938) believes that drainage from land contributes to the phosphate content of the North Sea and the Channel. Delsman (1939) believed that there is not much addition of nutrients to the sea from land drainage. Seshappa (1953) states that some phosphate is also brought to the sea by the rivers on the west coast of India which flow over laterite soil as the laterite on analysis was found to contain phosphate.

In this connexion, some observations in estuaries are of interest. High phosphate content has been reported in marine estuaries by MacGinitie (1935) and Howes (1939); the latter author also gives some reasons for the relative richness of phosphate in the estuaries. Stephenson (1949 *a*, *b*) observed an increase in the phosphate content of the water in which estuarine muds had been shaken. Rochford (1951 *a*, *b*) has indicated an exchange of phosphate between the mud and overlying water in the Australian estuaries. He further states, however, that the assumption of persistent discharge of nutrients by rivers and estuaries into the adjoining neritic waters is not generally true for most of the Australian coast-line so far investigated. The importance

of the bottom muds in the phosphate cycle has also been stressed, particularly in the freshwaters, by Mortimer (1941, 1942), Hayes *et al.* (1952) and Hayes (1955).

In the area of the present investigation analyses of the water of the Korapuzha estuary (a few miles north of Calicut) showed (George, 1953 *b*; Mr. S. V. S. Rao, oral communication) that the phosphate content of the water was generally of a low order during the south-west monsoon floods; hence, it would appear that there is not any significant addition of this nutrient to the sea from the land drainage here on the west coast (*cf.*, however, p. 236, below). The causes for the considerable increase of phosphate in the sea during the south-west monsoon season have to be sought elsewhere; and, as in the case of estuaries, the bottom muds are of interest.

Moore (1930, 1931) who investigated the muds of the Clyde Sea area found phosphate and nitrate in the mud; the phosphate values fell off with increasing depth in the mud, but usually showed a rise at the 10–15 cm. level; and nitrogen values usually fell with increasing depth. In the English Channel, Armstrong and Harvey (1950) attribute the changes in the integral mean concentration of phosphate to different water masses of different total phosphorus concentration passing through the area, rather than to a seasonal change in the total phosphorus content of a water mass. No marked deposition of phosphorus containing detritus was found by them; however, they noticed that deposits well offshore were not devoid of phosphate, some of which may dissolve in the water in the course of years. Hendey (1951, p. 14) reports that water expressed from the mud banks gives high figures for phosphate and silicate and that these nutrients show a rise in the concentration with falling tide due possibly to turbulence set up by the ebb tide bringing the nutrients out of the sand and mud banks. Miller (1952) who analysed phosphorus in the water and bottom sediment in the Biscayne Bay, Florida, found that a *decrease* of phosphorus in the water was associated with an *increase* in the sediment and *vice versa*. Ingle, Ceurvels *et al.* (1955) have shown that the muds of Mobile Bay are rich in phosphorus and also contain nitrogen in a form that can be used directly or indirectly by plant life.

Seshappa (1953) and Seshappa and Jayaraman (1956) investigated the bottom muds of the inshore area very near the sampling stations of the present investigation. They found that the values for interstitial phosphate were higher than corresponding values for inorganic phosphate in the overlying water during the pre-monsoon months; that the mud here retains relatively large quantities of phosphate and perhaps other nutrients. It is further stated that the mud is of laterite origin and an analysis of the sample of

laterite has revealed high " adsorbed " phosphate. Another cause for the increase of phosphate during the south-west monsoon months is attributed by them to the death and decay of bottom fauna at the commencement of the season. Observations by the writer during the past seven years, however, have shown that large-scale mortality is not a regular feature every year on this coast to be construed as a source for phosphates in the enrichment of the waters. It may be mentioned here that Du Cane *et al.* (1938, pp. 42–43) mention phosphoric anhydride in their analysis of laterite and mud from the west coast of India (*see* p. 231 of this paper).

It would, therefore, appear that in the present area, considerable quantity of phosphate is locked up in the sea bottom mud and this goes into solution during the stormy weather such as during the south-west monsoon season or during periods of high wind velocity (as observed by Moore, *l.c.*, phosphate content in the bottom mud in the present area also may be higher slightly beneath the surface of the mud than at its surface and only a strong agitation of the water affecting the mud as well will bring the phosphate into solution) and become available in plenty for phytoplankton growth; and, the concentration of the same remaining high till about September is very probably due to the quick regeneration as well of the phosphate into the water as suggested by Cooper (1935 *c*), Seiwell and Seiwell (1938), Renn (1937), Gardiner (1937) and Waksman, Hotchkiss, Carey and Hardman (1938). Seiwell and Seiwell state that the rate of liberation of phosphate both from sea-water and from decomposing plankton by the growth of the organisms associated with the decomposition exceeds the rate of consumption. There may also be considerable addition by the upwelling of deeper waters during this season. These may explain the high concentration of phosphate in the waters of the west coast during the bloom of phytoplankton and, obviously, there is more phosphate in the water than is required for the growth of the floral elements; hence, the high standing values. Later on, when calmer weather prevails, the dying and dead elements of plankton would sink and decomposition of them takes place *at the bottom* and the phosphate thus liberated remains " stored up " in the bottom sediment while the concentration of the same in the water diminishes. The stored up phosphate would go into solution and become available for phytoplankton growth again when stormy weather conditions occur next, particularly during the south-west monsoon. Thus, on the west coast of India, as shown by Miller (1952) elsewhere, a cyclical seasonal exchange of phosphorus between the sediment and water seems to occur, a decrease of phosphate in the water meaning an increase of same in the sediment and *vice versa*; and, this is confirmed for the west coast by Seshappa (1953) and Seshappa and Jayaraman (1956).

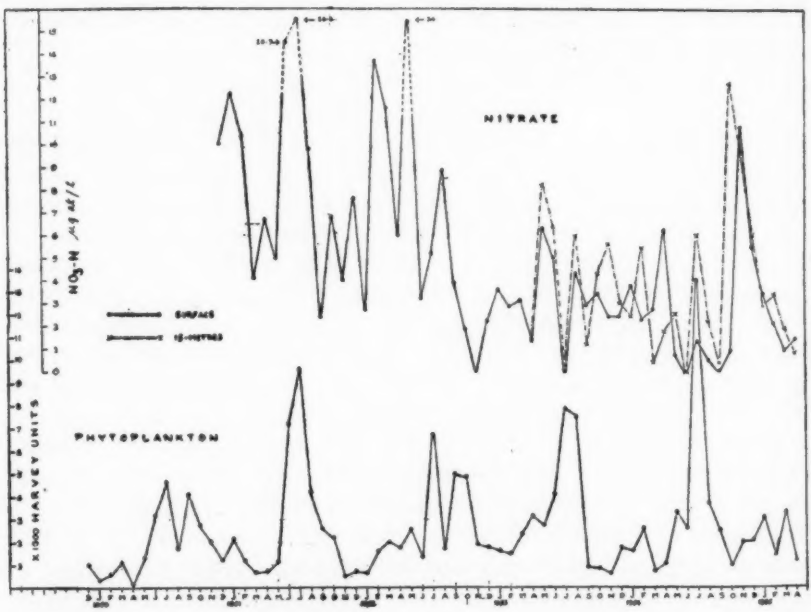
It was pointed out in the beginning of this section that, on some occasions, the concentration of phosphate is greater at the surface than in the bottom water. This is perhaps due to the greater regeneration in the surface layers by a shortened cycle also, through the fæcal pellets of zooplankton resulting from their feeding profusely on the phytoplankton and lack of mixing up of the water layers.

Finally, it may be stated that the phosphate content recorded indicates a high fertility rate for the waters on this coast.

(*b*) *Nitrate-Nitrogen*

Investigations on nitrate content of surface waters cover a period of just over four years during two years of which the bottom samples were also studied. The results are shown in Text-Figs. 4 and 7.

The concentration of nitrate is characterised by frequent rise and fall. Generally, the bottom values are slightly higher than surface values but on some occasions the latter exceeded the former. Higher concentrations of nitrate in the water are recorded during the pre-monsoon months of April



TEXT-FIG. 4. Seasonal fluctuation in the nitrate content of the water, surface and bottom and of phytoplankton (Harvey Units). Explanation in text.

or May and the main peak also occurs during this period or in the early part of the south-west monsoon. In 1954, however, the main peak occurred in October. In addition to the major peaks occurring during April, May, June or July, peaks of the second order occur during November or in some years in February (Text-Fig. 7).

The nitrate content of the water goes up to even 34 μg. at./L.; but, generally, the values do not exceed 13 μg. at./L. The values fluctuated between 2·37–26·2 μg. at./L. in 1951–52; 1·40–34 μg. at./L. in 1952–53; 2·25–6·27 μg. at./L. in 1953–54; and 0·47–10·75 μg. at./L in 1954–55 at the surface. At the bottom, the values were 0·43–8·20 μg. at./L. in 1953–54 and 0·36–12·71 μg. at./L. in 1954–55. The overall average for the water column ranged between 2·146 and 9·67 μg. at./L. On the whole, the concentration of nitrate in the water in 1951 and 1952 was of a higher order than in the succeeding years till October 1954. There appears to be an abundant supply of this nutrient as at no period depletion was recorded though occasionally in a few individual samples no nitrate could be detected.

No separate estimation of nitrite was made but the values for nitrate include that for nitrite as well, as both give the same colour reaction with reduced strichnine (Harvey, 1945). A few analyses made since June 1955, by the writer, however, show that nitrite is absent most of the time in the surface water, but present in small quantities in the bottom samples. Its concentration varied from 0–0·025 μg. at./L. at the surface and 0–1·25 μg. at./L. at the bottom (*see* also Bal *et al.*, 1946; Jayaraman, 1951; Ramamurthy, 1953 *b*).

There are not as many accounts of nitrate estimations as there are for phosphate. Some of the accounts available are shown in Table III for comparison.

Compared with the nitrate values of certain rich phytoplankton-producing areas in the temperate regions, the sea off Calicut, as in the case of the phosphate, has fairly abundant quantity of nitrate and hence nitrate could not possibly be a limiting factor in the production of phytoplankton here.

The nitrate content attains its maximum during the south-west monsoon season; in fact, high values (Text-Figs. 4 and 7) are recorded even prior to the onset of the monsoon which remains more or less stationary even when enormous production of phytoplankton is taking place showing thereby that there is a continuous supply of this nutrient to the water and the recorded value is the "standing" nitrate content of the season. Peaks of lesser intensity follow in November and February.

TABLE III

	Region	Depth in metres	Conc. NO_3—N in μg. at./L		Reference and remarks
			Lowest	Highest	
1.	Indian Ocean ..	1000		Circa 38·0	Sverdrup *et al.*, 1942 (value from figure)
2.	Arabian Sea ..		20·0	35·0	Gilson, 1937
3.	Bay of Bengal ..		8·0	16·0	Jayaraman, 1951
4.	Gulf of Mannar ..		0·0	5·0	Jayaraman, 1954
5.	Calicut—West Coast of India	Surface	0·47	34·0 (rare; Circa 20 common)	Present investigation
6.	Calicut—West Coast of India	15	0·77	12·71	do.
7.	Atlantic Ocean ..			Circa 30	Sverdrup, *et al.*, 1942
8.	English Channel			8·0	Cooper, 1937 *b*
9.	Pacific ..			Circa 35	Sverdrup *et al.*, 1942
10.	Friday Harbour ..			25	Phifer and Thompson, 1937
11.	New South Wales Coast	Surface		3·5	Dakin and Colefax, 1940
		45·7 metres		9·5	
		160 metres		10·7	
12.	Barents Sea ..		14	22	Kreps and Verjbinskaya, 1930
13.	Antarctic ..		28	43	Ruud, 1930

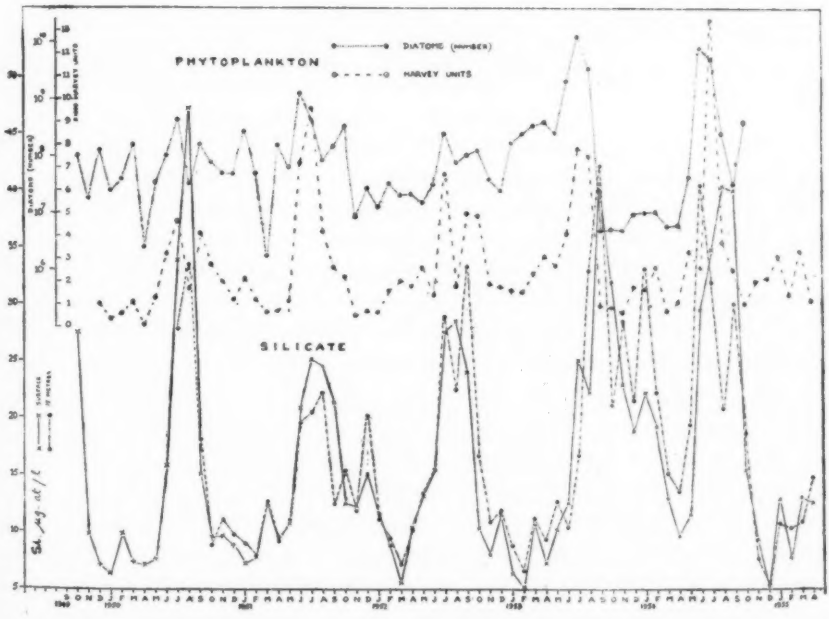
Moore (1930, 1931), Rakestraw (1933) and Ingle *et al.* (1955) have given evidences for the presence of higher concentration of nitrates in the bottom mud and water layer immediately overlying it. While one does not know the contribution made by river influx to the nitrate content of the sea around Calicut, it is possible that the bottom sediments here may similarly have considerable quantity of nitrogen compounds which are liberated into the overlying water in an assimilable form (*see* also Gilson, 1937, pp. 59–69) during the monsoon season in addition to contribution by upwelling in much the same way as the phosphates are replenished.

(*c*) *Silicate-Silicon*

The bulk of the phytoplankton in the sea is contributed by the Diatoms. Silica is essential for their growth and a lack of it acts as a limiting factor (Richter, 1904, 1906; King and Davidson, 1933; Meloche *et al.*, 1938; Gardiner, 1941; Denffer, 1949; Lund, 1950; Goldberg *et al.*, 1951; Jørgen-

sen, 1952, 1953; Wawrik, 1952; Komarowsky, 1953). A shortage of silica is known to slow down Diatom growth in the Antarctic (Hart, 1934, 1942; Clowes, 1938). Individual Diatom species too have shown a similar behaviour in varying concentration of silica (Hart, 1934; Braarud, 1948; Lewin, 1955 *a*).

The results of silicate estimations are presented in Text-Figs. 5 and 7. It will be seen that most of the time the bottom values for silicates are slightly higher than those for the surface, but on some occasions, the south-west monsoon period, the surface value often exceeds that of the bottom layers. The silicate content is at its maximum during the south-west monsoon period, July to September. Peaks of diminishing intensity occur during the following months. The lowest values are recorded between January and April. The silicate content never goes below 5 μg. at./L.; usually, it oscillates between 8 and 15μg. at./L. during the north-east monsoon months, and during the south-west monsoon months, the values commonly vary from 20–27 μg. at./L. (occasionally up to 47·22 μg. at./L.).



TEXT-FIG. 5. Seasonal fluctuation in the silicate content of the water, surface and bottom and of phytoplankton (Harvey Units) and Diatomaceæ (numbers), the silica consumers. Explanation in text.

The annual variation in the concentration of silicate-silicon in μg. at./L. was respectively as follows in the years 1950–51 to 1954–55 (May–April): 7·34–47·22; 5·60–25·08; 5·18–28·53; 9·64–40·58; 5·17–40·43, at the surface; and 8·33–54; 7·17–22·22; 6·167–33·33; 10·42–42·24; 5·30–40·47, at the bottom.

Silicate concentrations noted here are generally considerably higher when compared with similar records from other areas. Silicate values range, in terms of μg. at./L., 0·167–7 in the English Channel (Atkins, 1926 *a*; 1928, 1930, 1953; Cooper, 1933 *a*, *b*, *c*; Armstrong, 1954, 1955); from 0·0668–1·67 in the Great Barrier Reef region (Orr, 1933); 5·25–32·55 in the Bombay Harbour (Bal *et al.*, 1946); from 2–22 in the Bay of Bengal (Jayaraman, 1951; Ramamurthy, 1953 *b*); and 3–30 in the Gulf of Mannar and Palk Bay (Jayaraman, 1954). In the Bay of Biscay, even at a depth of 3,000 metres, a silicate value of 23 μg. at./L. only is reported (Atkins and Harvey, 1926). In the Antarctic (Clowes, 1938) and Pacific (Barnes and Thompson, 1938; Thompson *et al.*, 1934) the silicate values are very much greater, 117–170 μg. at./L. respectively. The values recorded in the present investigation are quite comparable with most of those mentioned above. There is always an abundance of silicate for Diatom growth in the waters of the west coast, the minimum value recorded here being comparable with the maximum in the English Channel.

There is an inverse relationship between the Diatom content in the water and silicate concentration, particularly during the period October to April following, on a sample-to-sample basis (*see* also Lund, 1950; Iyengar and Venkataraman, 1951); but owing to a continuous replenishment or addition of silicates during the south-west monsoon months, such a relationship between the Diatom population and silicates is obscured. It is to be noted here, however, that silicate concentration attains its maximum only after the Diatom bloom has started to wane (Text-Figs. 5 and 7).

The exact source and mode of this continuous replenishment of silicate is not known. However, it may be mentioned here that various views have been expressed on the silicate source. A terrigenous origin of the silicates is suggested by Atkins (1927). According to him, soils and disintegrating rocks part with silicate to the water readily and the silicate content of the water is most likely derived from dispersed clay than from Diatom skeletons whose rate of solution is too slow to matter. Hart (1934), Cooper (1933 *b*) and Harvey (1955) have shown evidences of a certain amount of solubility of the siliceous Diatom frustules, while a major portion is generally insoluble (Coupin, 1922; Conger, 1941; Rogall, 1939; Atkins, 1945).

A great deal has to be said towards the locking up of a considerable quantity of the silicate in an insoluble form in the Diatom frustules leading to the formation of large fossil deposits at the bottom. Still one has to mention that there are many Diatom genera which have weakly silicified frustules (or walls). These are not represented at all or very scarcely represented in the deposits (*vide* Karsten, 1928; Small, 1945; Kolbe, 1954, 1955, 1957). Some of these weakly silicified Diatom genera are known to form extensive blooms. It is probable that the weak nature of the silicified wall might be responsible in the same way to bringing back a part of the silicate from the Diatom detritus.

Apart from the above factors, there is a lot of available silicate in the bottom mud in the sea on the west coast of India which goes into solution into the water layers above when the sea bottom is agitated by wind action. It is also probable that some quantity is derived from the influx of river-water into the sea during the south-west monsoon season as many estuarine areas are known to have high silicate values (MacGinitie, 1935; Orr and Moorehouse, 1933; George, 1953 *b*; Iyengar and Venkataraman, 1951). Cooper (1952 *b*, p. 579) states that the drainage from the Tundras of the Northern Hemisphere should yield very large quantities of silicate to the North Polar Sea. Hendey (1951) has stated that water expressed from mud banks gave high values for silicate and also for phosphate. These investigations show the importance of bottom sediment in the silicate cycle.

In connexion with the silicate cycle, it may be mentioned here that, at the time of the occurrence of intense blooms of *Noctiluca* with *Protoeuglena noctilucæ* Subrahmanyan as a symbiont (referred to below as *green-Noctiluca*), Subrahmanyan (1954 *a*) found that Diatoms were almost absent in the water and silicate content of the water had gone up considerably even comparable with the high values obtaining during the south-west monsoon months. The salinity during the occurrence of this *Noctiluca* varied from 33–35·4‰, contrary to Delsman's (1939) observation in the Java Sea. Prasad and Jayaraman (1954) also found during the swarming of *green-Noctiluca* in the Palk Bay and Gulf of Mannar, high silicate concentration compared with the usual concentration and at that time the salinity also was high owing to the influence of the waters of the Indian Ocean and Arabian Sea. Usually, an occurrence of high silicate is associated with a fall in the salinity caused by admixture of river-water. They, therefore, attribute the high concentration of silicate to the active regeneration *in situ* of silicate from the frustules of Diatom by " comminution " as suggested by Cooper (1952 *b*) and, that such a change may be taking place during the active feeding of Diatoms by the *Noctiluca*.

The rôle of biological flora in the replenishment of the silicates has been stressed recently in some papers. Cooper (1952 *b*) has suggested that breaking up of the Diatom frustules in the guts of animals help to a certain extent in exposing fresh surfaces for action of water in dissolving silicates. The rôle of enzymes in this silicate regeneration has been earlier stressed by King and Davidson (1933). Recently Prasad and Jayaraman (1954) suggested that silicates from Diatoms are made available (soluble) by *Noctiluca* eating them, in much the same way as was suggested by Cooper and it is to this process that these authors attribute the high silicate value that accompany these blooms of *Noctiluca*. While Cooper suggested fracturing of Diatoms in organisms as enabling such a regeneration, the process by which these Diatoms are rendered soluble by *Noctiluca* is yet to be known, whether enzymes of *Noctiluca* have any rôle in this is yet to be worked out.*

Copepods have been known to occur in plenty simultaneously with *Noctiluca* though not in the same swarm or patch. The fæcal pellets of these Copepods have in them broken frustules of Diatoms (*see* Part I for relationship with types of Diatoms). Similar fractured frustules have also been found to occur in many instances of feeding on Diatoms. Thus the rôle of biological replenishment of silica may involve many more feeders than any single organism.

In fact, the silicate concentration shows little or no relationship to the bloom organism (refer Table IV). Notwithstanding the specific nature of the bloom, there is generally a complete eclipse or elimination of the Diatoms in that water mass (*see* p. 213 and below ; Prasad and Jayaraman, 1954) and in the neighbouring water mass too the Diatom population is minimal. Thereby, one is prone to conclude that the appearance of a monospecific bloom of an organism more or less affects the environmental conditions for the growth of Diatoms and probably causes its disappearance. Thus, there seems to be a greater degree of inverse correlation between silica content and silica consumers, the Diatoms.

A somewhat inverse relationship obtains between the silicate content and salinity (Text-Figs. 2 and 5; *see* also Jayaraman, 1954). But, this relationship is upset during monospecific blooms of organisms discolouring the water, such as *green-Noctiluca, Hornellia marina* (Subrahmanyan, 1954 *a, b*) and a palmelloid Chrysophyceæ (Subrahmanyan, unpublished), when high

* *See* also Prasad (1958). The majority of the genera reported within *Noctiluca* are strongly silicified representatives which are known to occur in fossils and oozes; only three out of ten genera, *viz.*, *Rhizosolenia, Bacteriastrum* and *Chætoceros* are absent or scarce in deposits. *See* also p. 213.

TABLE IV

Year, Month, Date		Salinity ‰		Silicates μg. at./L.		Temperature ° C.	O_2 Percentage saturation	Diatomaceæ No. of cells in each vertical haul	Remarks
		Surface	15 metres	Surface	15 metres				
1949 Oct.	14								*Hornellia marina* occurred
	31	31·20	33·40	25·00	..	..	91·40	189 120 960	
Nov.	1								*Hornellia marina* present
	3								
	7	28·60	31·50	12·00	..	..	95·00	12 960	
	14	34·40	34·30	11·67	..	27·5	63·80	227 500	
	21	34·20	34·50	7·83	..	28·5	97·0	5 734 800	
	28	34·80	34·90	8·17	..	28·4	114·7	64 909 440	
Dec.	5	35·20	35·40	7·67	..	28·0	96·0	14 060 880	*H. marina* occurred
	7								*H. marina*
	12	34·20	34·20	6·00	..	27·5	89·0	257 598 720	
1950 Aug.	7	26·50	34·60	41·67	37·00	25·1	151·0	15 878 400	
	14	25·00	34·30	66·67	30·30	25·9	89·1	22 492 800	
	21	31·90	34·30	33·33	33·33	23·7	46·6	6 662 400	*H. marina* present
	28	31·30	33·60	..	..	27·0	105·9	69 480 000	
Sept.	4	32·10	34·20	14·17	16·67	26·3	74·8	29 068 800	*H. marina* in enormous numbers
	11	30·30	34·60	..	16·67	23·9	97·1	136 968 000	
	18	30·50	35·20	14·50	..	26·1	140·2	423 456 000	
	25	35·00	35·20	16·67	20·83	25·7	92·3	39 321 600	*H. marina* present
Oct.	16	33·60	34·80	8·17	7·50	27·1	79·7	166 560 000	
	23	32·10	32·20	11·17	12·50	28·4	116·4	46 402 560	*H. marina* present
	30	33·20	34·90	7·50	10·00	27·8	103·0	116 966 400	*H. marina* present

TABLE IV (*Contd.*)

Year, Month, Date		Salinity ‰		Silicates μg. at./L.		Temperature ° C.	O_2 Percentage saturation	Diatomaceæ No. of cells in each vertical haul	Remarks
		Surface	15 metres	Surface	15 metres				
Nov.	6	33·20	35·60	12·50	15·83	27·6	110·3	147 363 840	*H. marina* present
	13	32·00	34·30	7·50	9·00	28·2	128·4	15 605 760	*H. marina* present in good numbers
	20	34·20	34·00	9·50	10·00	28·5	161·8	13 017 600	*H. marina* present in considerable numbers
	27	35·00	35·20	8·83	9·50	28·2	114·1	20 044 800	*H. marina* present in considerable numbers
April	2	35·00	35·00	11·67	10·83	29·3	79·8	51 600	
	9	35·80	35·60	14·17	13·33	29·3	88·3	326 400	
	14								*H. marina present*
	16	34·50	34·90	6·67	6·00	29·0	108·0	694 517 760	
	18							182 928 000	*H. marina* present
1951 Aug.	20	32·30	34·80	22·21	22·21	26·0	114·4	60 160 000	*H. marina* present
	27	30·40	33·80	23·80	27·67	23·0	47·8	95 942 000	*H. marina* present
Sept.	3	34·40	34·60	16·67	..	25·2	29·9	23 592 000	
	10	33·90	34·00	37·03	16·67	26·0	98·8	388 224 400	*H. marina* appeared in good numbers
	17	34·20	34·70	22·21	..	25·1	45·9	53 742 000	*H. marina* appeared in good numbers
	24	33·60	34·20	9·17	8·33	28·6	87·6	125 964 000	*H. marina* present
Oct.	3	30·80	35·10	12·50	16·67	28·1	95·6	1585 440 000	
	10	34·60	34·90	..	..	28·0	111·5	23 184 000	*H. marina* in large numbers
	15	26·80	35·20	16·67	..	28·9	106·2	40 137 600	*H. marina* in large numbers

	22	34·60	35·00	8·33	14·20	28·7	114·8	7 440 000	*H. marina* in large numbers
	29	32·20	34·00	..	..	30·0	114·2	1 593 600	*H. marina* in large numbers
Dec.	3	31·50	34·00	10·83	10·83	28·5	111·0	129 561 600	
	10	34·50	35·00	13·33	16·67	28·6	64·4	739 200	*H. marina* present
	17	34·00	34·40	23·80	47·62	28·4	84·9	16 014 000	
	24	34·80	35·10	13·33	13·33	27·8	90·0	3 074 400	
	31	35·20	34·60	14·17	12·50	27·9	85·3	148 800	*H. marina* few present
1952 Aug.	18	32·20	34·00	7·50	12·00	24·8	95·7	249 062 400	
	25	31·20	34·90	20·83	22·17	24·1	90·8	2 313 600	
Sept.	1	34·40	34·80	16·67	..	25·0	87·9	67 929 000	
	6								*Hornellia marina* made its appearance
	8	34·00	..	23·67	33·33	24·2	60·8	32 025 600	*H. marina* in abundance
	15	33·40	33·80	22·17	33·33	24·8	47·4	61 344 000	*H. marina* some dying
	22	34·00	35·00	33·33	33·33	24·3	52·7	282 816 000	*H. marina* some dying
	29	34·20	34·80	..	..	25·9	50·8	72 307 200	
Oct.	6	32·60	34·20	9·17	16·67	27·2	78·7	106 629 120	
	13	21·80	33·40	..	..	27·8	86·8	1 502 000	
1953 Sept.	2	..	..	..	..	26·3	55·2	2 995 000	
	7	30·20	34·40	41·67	36·67	24·9	83·7	249 600	*H. marina* present
	14	25·80	34·20	59·60	60·60	..	..	1 670 400	*H. marina* increased a little
	21	34·20	34·20	6·06	16·67	25·2	..	15 494 400	
	26								*H. marina* reappears in large numbers
	28	34·60	34·00	55·00	55·00	25·2	..	4 915 200	*H. marina* blooms dis-
Oct.	5	28·20	35·00	41·67	16·67	..	..	9 100 000	colouring water. Bloom
	12	32·40	34·20	16·67	16·67	..	..	806 400	*continued* till Oct. 5,
	19	30·80	33·20	50·00	41·67	..	..	3 110 406	1953. Later part of
	26	30·00	33·20	19·50	19·50	..	94·5	8 928 000	the period of bloom
Nov.	2	32·60	34·60	20·33	30·33	28·7	77·2	283 000	with dead and decay-
	9	34·60	34·80	30·33	41·67	28·4	93·6	7 641 000	ing elements in the

TABLE IV (*Contd.*)

Year, Month, Date		Salinity ‰		Silicates μg. at./L.		Temperature ° C.	O_2 Percentage saturation	Diatomaceæ No. of cells in each vertical haul	Remarks
		Surface	15 metres	Surface	15 metres				
	16	34·00	34·20	20·83	19·50	29·0	105·5	11 040 000	water. Data for O_2
	23	32·60	32·60	22·16	30·16	29·0	94·3	..	not available (lost);
	30	31·80	32·00	20·83	20·83	28·6	..	633 600	however it may be
Dec.	7	32·20	32·20	20·83	25·00	28·5	88·9	8 486 000	mentioned that O_2
	14	32·60	32·60	16·67	23·66	28·5	83·7	11 788 000	content was at or
	21	31·20	31·20	16·67	16·67	28·1	..	3 590 000	above saturation level
	28	32·00	32·00	20·83	20·83	28·4	90·5	14 899 000	most of the period except towards the end when values fell with increase in dead organic matter in the water. Note high silicate values. After Oct. 10, the alga waned.
1952 Nov.	3	34·80	34·80	9·33	9·83	28·0	72·1	64 944 000	
	10	34·00	34·20	8·33	13·33	27·8	116·9	57 216 000	
	17	34·80	34·80	5·83	5·83	27·2	119·5	21 120 000	
	24	34·40	34·60	8·33	14·70	28·5	107·6	998 400	
Dec.	1	34·20	34·20	12·50	9·17	27·9	75·7	34 368 000	
	8	34·40	34·70	20·83	22·17	28·2	93·6	82 252 000	*Green Noctiluca* began
	15	33·70	33·70	8·33	12·50	28·7	97·6	10 732 800	to appear and
	22	32·20	32·50	7·50	7·50	28·1	97·7	2 323 200	occurred in plenty
	29	31·30	32·00	8·33	8·33	28·3	90·4	2 899 200	from 10–13, Dec. 1952

1953 Mar.	2	34·60	34·60	4·17	4·17	29·0	85·9	1467 360 000	and continued till end of the month.
	9	34·40	34·60	..	8·33	29·8	91·4	223 296 000	
	16	34·60	34·70	14·17	16·67	29·0	67·6	7 220 000	
	23	34·60	34·80	14·17	16·67	29·0	67·6	7 220 000	
	30	34·10	34·60	10·00	10·00	29·6	87·0	1 560 000	*Green Noctiluca** reappeared and began to increase quickly and discoloured the water. Note sharp fall in number of Diatoms and increase of silicate value.
1953 April	6	34·80	34·90	9·17	9·17	29·7	92·5	121 800 000*	Bloom continues
	14	35·00	35·00	6·67	10·00	31·4	94·8	1032 480 000*	Fewer numbers
	27	35·20	35·40	6·67	10·00	28·5	74·4	6 288 000*	In abundance
May	4	35·50	35·60	12·50	16·67	28·5	69·6	2 856 000*	In abundance
	11	35·60	35·60	16·67	16·67	28·8	73·6	1 128 000*	In abundance
	18	35·40	36·00	8·33	12·50	27·8	65·2	12 192 000*	Diminishes in number
	25	35·40	35·80	3·33	5·00	28·4	97·7	1027 008 000*	Nil
June	2	35·20	35·80	12·50	12·50	27·4	52·13	686 496 000*	Few numbers
1955 Mar.	7	34·20	34·30	17·40	13·50	30·0	81·9		Palmelloid chrysophyceæ appears. Most abundant bloom on 14th and 15th March, discolouring the water. Diatoms practically nil in that water.
	14	34·40	34·40	6·00	7·50	29·5	106·6		
	21	34·60	34·60	13·50	13·50	30·0	82·8		
	28	34·90	34·70	15·00	9·00	30·5	83·2		

silicate values were recorded coincident with higher salinity values (refer Table IV). This is to be attributed, again, to the scarcity or absence of the Diatoms from the water mass owing possibly to the deleterious effect caused by the organism concerned as suggested by the work of Lucas (1947, 1949, 1955), Rice (1954) and Provasoli and Pinter (1953 *b*).

(*d*) *The Phosphate-Nitrate-Silicate Ratio*

The fluctuation in the nitrate content is so erratic that the ratio of P to N in the sea-water on the west coast of India, at any given time, varies very much as in other regions (Harvey, 1945, 1955; Phifer and Thompson, 1937; Redfield, 1934, Cooper, 1938 *b*). Nevertheless, over long periods, there is a constancy in the ratio of these elements in the water in the seas. When the overall average obtained here over some years is worked out, the ratio in terms of μg. at./L. (with P-value corrected for salt error by $\times 1 \cdot 12$*) (Cooper, 1938 *b*) was found to be P: N: : 1: 15, the same as has been found in the temperate regions (Cooper, 1937 *a*, 1938 *a*, *b*; Redfield, 1934; Sverdrup *et al.*, 1942, pp. 236–37).

Fluctuations do occur in the ratio of silicate-Si to phosphate-P, though not to the same extent as in the case of phosphate-P and nitrate-N. The ratio of silicate-Si to phosphate-P in terms of μg. at./L. was found to be 20: 1, when based on an overall average of five years' observations. The same ratio is indicated in the temperate region also (Sverdrup *et al.*, 1942, p. 261).

Therefore, the ratio between these elements Si, N and P is 20: 15: 1 which is the same found in other regions where intense work had been done. This resemblance to the temperate regions is interesting and is possibly an indication of the uniformity and equilibrium in the content of these elements in most marine environments.

As observed by Armstrong and Harvey (1950, p. 145) the water mass sampled on each occasion is not the same, but a series of observations taken at one position for a number of years will iron out differences arising from changing water masses. Thus it is evident that the differential requirements of these elements by the individual biological components in the water might bring about temporary anomalies as seen in the fluctuation of the ratios (*see* Redfield, 1934). One has only to support Armstrong and Harvey on the need for studies ranging over long periods as such studies alone can iron out differences arising from studies of individual water samples and bring out ultimately a general pattern characteristic of each water mass and region.

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* 1·15 according to Robinson and Thompson (1948).

In this connexion, the remarks of Redfield (1934, pp. 189–91) made with particular reference to phosphate and nitrate may be quoted here: "It appears to mean that the relative quantities of nitrate and phosphate occurring in the oceans of the world are just those which are required for the composition of the animals and plants which live in the sea. That two compounds of such great importance in the synthesis of living matter are so exactly balanced in the marine environment is a unique fact and one which calls for some explanation, if it is not to be regarded as a mere coincidence. It is as though the seas had been created and populated with animals and plants and all of the nitrate and phosphate which the water contains had been derived from the decomposition of this original population.... Whatever its explanation, the correspondence between quantity of biologically available nitrogen and phosphate in the sea and the proportions in which they are utilised by the plankton is a phenomenon of the greatest interest." Further, "the general agreement indicates that the amounts of nitrate and phosphate present in the sea are controlled in the main by the same agency—the consumption by the phytoplankton and regeneration from its remains" (*see* also Harvey, 1928 *a*, 1955, p. 28). This constancy has been used elsewhere (Part I) as a basis for the assessment of the standing crop for comparison with other areas as the ratio of these factors in plankton organisms show a constancy by weight (Fleming, 1940).

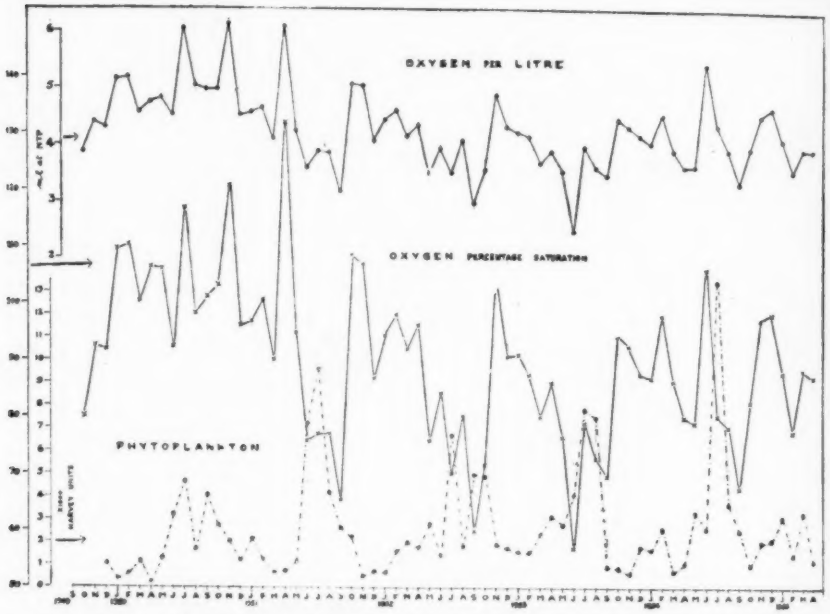
V. Dissolved Oxygen

The results of oxygen estimations are shown as monthly averages in Text-Fig. 6. Only surface values were being determined but some data for bottom samples are available for a short period since July 1954, estimated by the writer.

The monthly averages for dissolved oxygen per litre of sea-water varied from 4·10–6·11 c.c. in 1950–51; 3·18–5·06 c.c. in 1951–52; 2·97–4·88 c.c. in 1952–53; 2·48–4·55 c.c. in 1953–54; and 3·34–5·43 c.c. in 1954–55. There were more instances of supersaturation of the water in 1950 than during the following years. The oxygen content, generally, keeps fluctuating between 3·5 and 5 c.c. per litre.

The values for bottom samples, on most occasions, are lower than those for the surface water; but, in a few rare instances, the bottom samples showed more dissolved oxygen, particularly when sampling was done in the day, at about 10–11 a.m. as on offshore collection days. This may be due to the rise in the temperature of the surface water later in the day. Normally, the lower oxygen content of the water near the bottom is, perhaps, due to

the very rapid oxidation of the sediment and the slow diffusion of the dissolved gases as suggested by Brouardel and Fage (1955; also refer pp. 224–25 below).



TEXT-FIG. 6. Seasonal fluctuation in the oxygen content of the water ml./L. and its percentage saturation, and of phytoplankton (Harvey Units). Explanation in text.

The distribution of dissolved oxygen has been studied intensively in several geographical regions (*cf.* Sverdrup *et al.*, 1942; Harvey, 1928 *b*, 1945 and 1955). For comparison, the values recorded in some select regions may be cited here as it is not possible to refer to all the accounts from various regions.

In the English Channel, Cooper (1933 *b*) found the oxygen content increasing rapidly, reaching 8% supersaturation during the phytoplankton outburst. With rising temperature, degree of supersaturation fell. In the Great Barrier Reef region, Orr (1933) found the water 90–100% saturated and only once supersaturated. Only for a short period was saturation below 90%. At one station the range of saturation was 55–120%. Near Triumph Reef, Riley (1939) reports 99% saturation. At 15° S. in the Atlantic, Wattenberg (1929) found high oxygen content at the surface which extended to some depth below also. Smith *et al.* (1950) found that the oxygen content of the

water, at a sub-tropical inshore region adjacent to Miami, to be near saturation at all the stations away from pollution. McClenden (1918) reported that the sea-water in the shallow areas of Dry Tortugas was generally slightly supersaturated with oxygen during day and undersaturated by night.

In the Bay of Bengal, off Madras, the concentration of oxygen, according to Jayaraman (1951) and Ramamurthy (1953 *a*), varied from about 2·5–5 c.c. per litre; the latter found that the percentage saturation remained about 83–90%. In the Gulf of Mannar and Palk Bay (Jayaraman, 1954) there was no marked variation in the seasonal distribution and rarely did the water attain saturation with oxygen. The values ranged between 3·5 and 4·5 c.c. per litre. There was little difference between the surface and bottom values as the region concerned is shallow and vertical mixing takes place easily.

According to Harvey (1945, p. 46), the concentration of oxygen varies from 8 c.c. per litre in the Arctics to 4·5 c.c. in the tropical surface waters.

The *overall monthly average* of five years' data at Calicut shows an oxygen content variation of 3·88 c.c. per litre in March to 4·88 c.c. per litre in November; the percentage saturation varied from 72·68 in September to 105·91 in November. The values recorded here are very well comparable with those of other geographical areas cited above.

The seasonal fluctuation in the oxygen content of the sea-water at Calicut shows some interesting features. It is well known (Sverdrup *et al.*, 1942, p. 190) that solubilities of gases like oxygen and nitrogen which do not react chemically with the water or its dissolved salts decrease with increasing temperature and salinity. It is also well known that during the phytoplankton outburst oxygen concentration increases and even exceeds saturation point. Further, agitation of the water tends to increase the solubility of oxygen in it.

A study of Text-Figs. 1, 2 and 6 will indicate certain results, apparently, in contradiction of the points mentioned above. To begin with, high oxygen concentrations are met with in April, May or June, prior to or in the early stages of the bloom of phytoplankton. At this period, before the onset of the rains, temperature and salinity are both high; however, the effect of this is offset by the high wind force prevailing at this time which brings about agitation of the water leading to increased dissolution of atmospheric oxygen. There may, perhaps, be also water rich in oxygen brought in by the currents. It was mentioned earlier that there is an arm of the Antarctic flow surfacing in the Arabian Sea, and Antarctic waters are generally rich in oxygen content.

The south-west monsoon winds bring about an upwelling of these waters to the surface.

During the intense phytoplankton bloom in June–July, one would expect a further increase in the content of dissolved oxygen. In fact, there is slight increase compared with the earlier month. The concentration of oxygen at this time was between 3 and 4 c.c./L. only and the percentage saturation value was between 75 and 85%; In June 1954, however, the dissolved oxygen showed a supersaturation of about 6%. Similarly in 1950 a supersaturation of the waters was recorded. Salinity and temperature also at these times were in favour of a high dissolved oxygen content; but, curiously enough, there is a fall in the oxygen content after July even when the phytoplankton bloom is at its peak period. From September onwards, the oxygen content increases again.

Many factors have been held responsible for a lowering of the oxygen concentration. It is well known that while oxygen is liberated by plants during carbon assimilation during day, it is also consumed throughout day and night by both plants and animals in respiration. The phytoplankton bloom on this coast during June–July is very intense, and during this period, the number of zooplankters, particularly the smaller species, are also on the increase including reproductive stages of them with a greater demand on oxygen. Further, development of phytoplankton is accompanied closely by bacterial development (Waksman, Reuzer, Carey, Hotchkiss and Renn, 1933) and, obviously, during the phytoplankton bloom, bacterial population increases and oxygen is also increasingly consumed by the bacteria (*see* also Nash, 1947, p. 169). So, it would appear that the demand on oxygen liberated during photosynthesis is so high as to leave back little to raise the overall level of the concentration considerably towards the saturation or supersaturation level. Moreover, at this period, plankton detritus considerably increases in the water. The dead and decomposing organic matter in the upper layers consume considerable quantities of oxygen (Seiwell and Seiwell, 1938) and tend to lower the oxygen content of the water. According to Stephenson (1949 *b*) there is a close correlation between the reduction in oxygen concentration of the supernatant sea-water and the rate of release of phosphate during regeneration from mud surface. According to Richards and Redfield (1954, p. 281) high organic content is equivalent to high oxygen demand and so contact with organic-rich bottom sediment might be held responsible for the oxygen minimum (*see also* Thompson, Thomas and Barnes, 1934). The bottom sediment on this coast contains considerable quantities of organic matter, " marine humus " as will be indicated later in the next section. It has been shown earlier that blooms develop following similar

regeneration of phosphate, etc., from the bottom and this regeneration has similar demand on oxygen.

Yet another possible reason for the apparent low concentration of oxygen is indicated by the recent contribution of Lewin (1955 *b*). Lewin found that the uptake of silicate by Diatom cells is an aerobic process and addition of silicate stimulates *respiration* of silicon deficient cells. The bloom period on the west coast is one of a rapid increase of Diatoms and it is very likely that more oxygen is consumed by the rapidly dividing Diatoms with more and more utilization of silica. When organisms concerned are not Diatoms, but other algæ, green algæ, the increase of oxygen has been far more than that recorded at the Diatom bloom period (Table IV). Instances are known from freshwater environments where high supersaturation of the water due to photosynthetic activities of the algæ have led to injurious effect on the faunal elements; perhaps, if a fair quantity of Diatoms were present it would act as a check on supersaturation as the Diatoms consume oxygen during silica assimilation also. Suffice it to say here, that the oxygen content recorded in the sea-water here is that which is in equilibrium after the interaction of all these factors, and the quantity recorded, 3–4 c.c./L. during the season of the phytoplankton bloom is not inconsiderable at all.

After the period of the main bloom, the standing crop still holds relatively a considerable quantity of phytoplankton and fewer number of zooplankters and less of decaying organic matter; further, the rate of multiplication of the Diatoms also appear to fall off. So, the oxygen content shows an upward trend and saturation levels are reached in November or so, very probably also due to the fall in the quantity of oxygen-consuming plankters, for, generally, in November, the plankton content is poor in the water. During this time, however, the effect of the reversal of the coastal current in the Arabian Sea—from a clockwise to an anticlockwise direction—the influx of water from the Bay of Bengal and the higher wind velocity also should not be overlooked. Thus, it is seen that the seasonal fluctuation of the oxygen content on this coast is peculiar and interesting, influenced by meteorological, chemical and physical factors and the biological content of the water.

Addendum.—Since this paper was completed (1956) for publication, an account by Kasturirangan (1957) on the seasonal fluctuation in the dissolved oxygen content of the surface waters of the sea in the same area as that of the present investigation has appeared based on four years' data which are more or less common to his as well as the present account for the

years concerned. This author, while discussing the fluctuation in the oxygen content, remarks about the peculiarities noticed (*l.c.*, p. 145): " positive correlation with temperature and salinity from September to November " and the puzzling feature in the " great diversity of behaviour exhibited by the oxygen-curve in the period May to September". According to him, while the low values for September could be accounted for by the fall in the phytoplankton production and increase of zooplankton, " the reasons for the subsequent rise in the values and occurrence of a peak in November are not altogether clear". One explanation sought out for the period May to September concerns the mud banks which are supposed to exercise an effect in the direction of reduced oxygen. (It must be noted here that mud bank formation is not a very regular feature every year—writer.) The author himself has pointed out that the investigations of the present writer on phytoplankton production might throw some light on a number of puzzling features noticed. The discussion on these aspects in this section of the present paper provides answers for several apparent contradictions noticed in the fluctuations of the oxygen content of the waters on the west coast of India.

VI. OCEAN CURRENTS*

No direct work has been done on this aspect; however, some points of interest may be mentioned here. The currents move the water masses; and, sometimes, within the same water mass, some small changes in the direction may occur over small areas, probably owing to the contour of the coast and/or of the ocean floor, often indicated by the drifting of the nets cast. Be that as it may, the information gathered through fishermen fully confirms the picture of the course of circulation described based on the Admiralty records (Subramanyam, Part I). It may be mentioned here that the coastal currents are likely to have the waters of the Cold Antarctic Flow (Carpenter, 1887) and of the Antarctic Intermediate Current (Mohamed, 1940), already referred to elsewhere. These are bound to be rich in nutrients as the latter especially (Mohamed, *l.c.*, p. 193) on the east coast of Africa is found to favour the production of phytoplankton.

From the constancy of the main pulses of the plankton over five years, it is fairly certain that the movement of water may have a seasonal rhythm and conditions over a large extent of the west coast of India are similar though the intensity of production of plankton and its time may show some slight variations. The water mass sampled on each occasion is not the same,

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* More details relating to this aspect will be presented in another account (Subrahmanyan, 1959).

nor is it so in chemical composition or biological content; the seasonal meteorological changes control the nature and movement of the water masses; but, still the overall picture of the seasonal fluctuation of the biological elements in the water remain almost identical year after year, though there may be differences in the intensity of production.

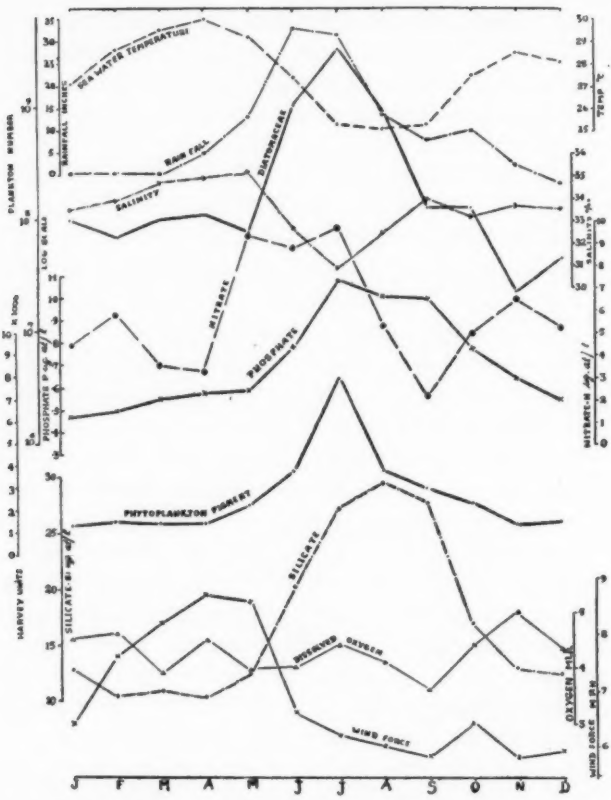
VII. General Observations on the Environmental Factors Affecting the Production of Phytoplankton with Remarks on possible Limiting Factors

To help understand the rôle of the environmental factors in the production of phytoplankton, the results of the investigation described in the foregoing pages may be briefly reviewed here and discussed. It may be borne in mind that the water occupying the area of investigation does not remain there stationary but is replaced by other water masses at intervals by currents, upwelling and other physical factors responsible for such changes; and, as such, the seasonal changes in a particular water mass cannot be determined directly. In the words of Armstrong and Harvey (1950, p. 145), " From observations at one position small seasonal changes may not be apparent and could be demonstrated only by a series of observations taken at one position throughout many years, sufficient for fluctuations due to changing water masses to iron themselves out. On the other hand, the considerable seasonal changes which occur in phosphate, nitrate, phyto- and zooplankton are quite apparent. They outweigh fluctuations arising from a succession of water masses passing through the area." These remarks appear to be equally applicable to the present area.

In Text-Fig. 7, the overall picture of the results presented in the earlier pages is shown based on the average of five years' data. This gives a clearer idea of the factors concerned in phytoplankton production dealt with in this account.

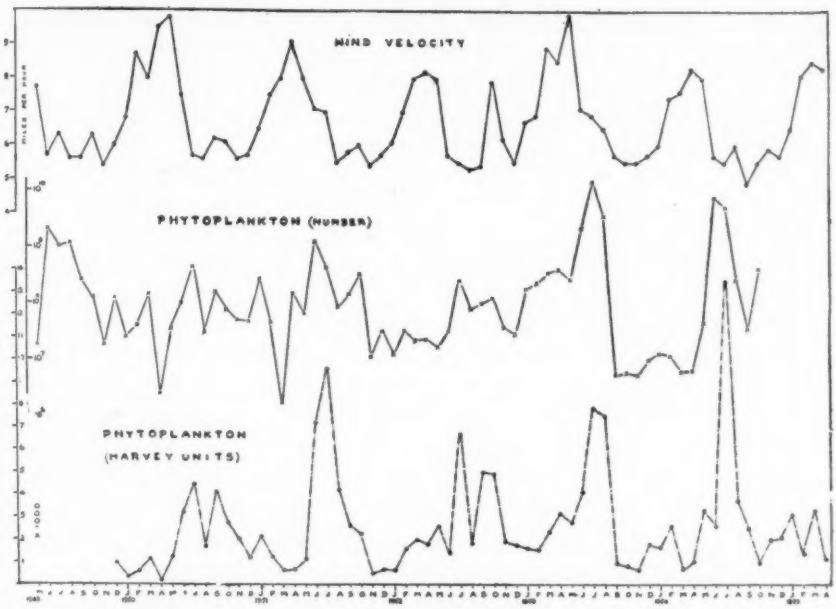
It has already been pointed out earlier that there is no lack of nutrient salts, such as phosphates, nitrates and silicates in the waters on the west coast of India, to act as a limiting factor; and that a fall in temperature to optimum levels (*circa* 25° C.) and that of salinity by 3–4‰ (to *circa* 30‰) during the south-west monsoon season brings about a bloom of phytoplankton (*see* pp. 193 & 200). This bloom is not sustained for long. However, phytoplankton crop again increases, irrespective of temperature and salinity values (*see* pp. 192 & 201) every time there has been a period of high winds.

A reference to Text-Fig. 8 will show that phytoplankton peaks *follow* immediately the peaks of wind force. In the area of the present investiga-



TEXT-FIG. 7. Seasonal fluctuation, based on overall average data of five years, of wind force, rainfall, dissolved oxygen, phosphate-P, nitrate-N, silicate-Si, salinity, temperature of the water, total phytoplankton (Harvey Units) and Diatomaceæ (numbers). Explanation in text.

tion, bottom-living forms like *Melosira sulcata*, *Navicula* spp., *Pleurosigma* spp. and several other pennate Diatoms were found to be common in the plankton samples, brought up to the upper layers, in addition to the increased development of the plankton forms, following periods of high wind velocity. In the Great Barrier Reef region, Marshall (1933, pp. 122–23) has found that wind force affects the depth distribution of the Diatoms and their total number also; there was a sharp rise of Diatom numbers on or just after the days of strong wind. Further, the general and lasting rise in Diatom numbers coincided exactly with the beginning of the South-West Trades. Not only the bottom-living pennate forms increased in number (also being brought up from lower levels to the surface) but non-pennate forms also increased



TEXT-FIG. 8. Seasonal fluctuation of wind velocity and of phytoplankton (numbers and Harvey Units). Explanation in text.

owing to greater growth consequent on the enrichment of the upper layers by the action of the wind (*see* also Cooper, 1952 *a*). Conversely, the Diatom numbers decreased when the South-East Trades began to slacken. Similarly, with fall in wind force the phytoplankton population diminished on the west coast of India also.

The effect of wind is not direct; obviously, the action of wind increases the supply of nutrients to the surface layers from the bottom layers, which acts as a stimulant to the growth of phytoplankton. In the present area, it has already been stressed that none of the usual inorganic nutrients, taking even the surface values alone, are deficient at any time in the water. Therefore, very probably, certain essential growth-promoting substances also seem to be liberated into the water from the bottom in addition to the phosphates, nitrates, silicates and so on. That there is an exchange of substances between the mud and the overlying water has been proved earlier. It appears that there is also some other (undetermined) substance, organic or inorganic, present in the mud which also is set free into the water, thus providing the stimulation for the growth of the standing crop of phytoplankton.

No investigation could be undertaken to determine the nature of this substance. Nevertheless, it may be mentioned that in cultures addition of soil extract, weed extract or organic substances such as thiamine, biotin, glutathinine, cystine, cobalamin (vitamin B_{12}), presence of trihydrol, or supply of certain elements like Fe, Zn, Mn, Mo, Co, etc., have an important rôle in the metabolism of algæ including the Bacillariophyceæ, the dominant element in the flora of the sea (Allen, 1914; Allen and Nelson, 1910; Barnes, 1932; Chu, 1943; Cooper, 1935 *a*, *b*; de Valera, 1940; Droop, 1954, 1955 *a*, *b*; Fogg, 1953; Gaarder and Gran, 1927; Goldberg, 1952; Gran, 1931, 1933; Gran and Ruud, 1926; Harvey, 1933, 1937 *b*, *c*, 1939, 1942, 1945, 1947, 1949, 1950, 1955; Hutner and Provasoli, 1953; Hutner, Provasoli and Filfus, 1953; Imamura, 1952; Iyengar and Subrahmanyan, 1944; Kylin, A., 1945; Kylin, H., 1942, 1943, 1945; Levring, 1945, 1946; Lewin, 1954; Marshall, 1933, Noack and Pirson, 1939, Pirson, 1937; Pirson and Bergmann, 1955; Pringsheim, 1946; Provasoli and Howell, 1952; Provasoli, McLaughlin and Pinter, 1954; Provasoli and Pinter, 1953 *a*, *b*; Robbins *et al.*, 1950 *a*, *b*; Rodhe, 1948; Schreiber, 1931; Subrahmanyan, 1947; Suneson, 1942, 1943; Sweeny, 1954). According to Iyengar and Venkataraman (1951) a high value for oxidizable organic matter favoured profuse growth of the Diatoms, *Nitzschia palea* and *Thalassiosira marginata*. In nature these substances occur in solution in the water and become available by various means, from bottom sediments, land drainage, melting ice or synthesis by bacteria (Ingle *et al.*, 1955; Johnston, 1955; Gran, 1929 *a*, *b*; 1930; Hart, 1934, 1942; Clarke, 1940; Nelson, 1947; Gilson, 1937; Grøntved and Seidenfaden, 1938; Føyn, 1929; Marshall, 1933). There is every reason to suppose that here also such substances occur in the bottom sediments in which decaying organic matter, the marine *humus* (Waksman, 1933) is present in abundance (*vide* also Anderson, 1939; Pillai, 1956) or are brought in by river influx during the season. According to Sewell (1952, p. 716) the bottom deposits in the Arabian Sea contain a high percentage of organic matter and this as well as other nutrients are brought up during the upwelling in the south-west monsoon season.

While considering the possible rôle of the bottom mud on the chemistry and biology of the waters on this coast, it may be interesting to know its composition in order to find out whether there is any evidence in it in support of the views given above. The mud of the mud banks on the west coast of India is believed to have been brought down by the present and past rivers and is mainly of a lateritic and alluvial formation and through them traceable to the decomposing gneissic rocks of the interior (Du Cane *et al.*, 1938). Laterite, from a chemical point of view, is a ferruginous clay, a lithomarge

containing variable quantities of ferric hydrate (Du Cane *et al.*, *l.c.*, pp. 43–44). The analysis of laterite as given by Du Cane *et al.* is given below:—

	Laterite*	Mud†
Volatile matter ..	12·41	14·40
Silica ..	46·84	44·05
Alumina ..	24·24	19·14
Ferric Oxide ..	13·53	4·45
Ferrous Oxide ..	1·27	3·64
Lime ..	0·42	6·18
Titania ..	1·38	Nil
Magnesia ..	Nil	5·34
Sulphuric Anhydride ..	Nil	2·26
Phosphoric Anhydride ..	0·02	0·38
TOTAL ..	100·11	99·84

* Laterite from the beach at Tellicherry, some miles north of Calicut.

† Mud from the mud banks of Narakkal, Calicut and Alleppey.

It is thus clear that the mud of the mud banks could be derived from the lateritic deposits. The data further show that the land drainage could be a good source of Fe, found so essential for the growth of phytoplankton, and of silica for the Diatoms in particular. Fe is important for the building up of chlorophyll and it is known that lack of it can be a limiting factor (Harvey, 1937 *b*). It is also evident that the mud is a storehouse of several other compounds and the presence of sulphur is interesting in that this element has been found to have a rôle in the silicon metabolism of Diatoms (Lewin, J. C., 1954; Harvey, 1939; Matsudaira, 1942). It may also be mentioned here, that waters rich in phosphate have also been found richer in iron (Cooper, 1948 *a*, p. 302, *b*; Armstrong and Harvey, 1950, p. 151). The rich phosphate content noted in the waters here may bear a relation to this fact.

The monsoon bloom of phytoplankton, in the absence of any other factor recognizable as a *trigger*, therefore, could be due to the stimulus provided by elements such as Fe, Mn and/or organic substances such as vitamin B_{12}. A study of the Fe-cycle here would be interesting, as also a detailed investigation of the bottom muds of this coast from all aspects, particularly, chemical and bacteriological. This would throw light on many problems including the fertility and production of organic matter in the sea on the west coast.

So far, the external factors, mainly the physical and chemical, influencing phytoplankton production were considered. The most important factor, however, is the physiological state of the organism concerned. Though this has long since been recognized, the *how* of it does not appear to have been dealt with.

Morphological and life-history studies (*e.g. see* Hustedt, 1930; Geitler, 1932; Fritsch, 1935; Iyengar and Subrahmanyan, 1944; Subrahmanyan, 1947) have shown that the Diatoms (they form the bulk of the phytoplankton in all seas) generally multiply by vegetative division, and after each division there is a diminution in the size of the cells and when the cell finally reaches a minimum size characteristic for the species, a process of sexual reproduction and auxospore-formation takes place and a new rejuvenated generation of actively dividing cells is produced which repeats the cycle. Experimental studies have proved that this process of sexual reproduction could be induced by a lowering (dilution of the culture medium) of the salinity of the medium within certain limits, provided the cells are of the right size. Studies of plankton samples reveal that auxospore-formation is mostly met with during the south-west monsoon period, the drop in the salinity also at that time favouring those species concerned to undergo sexual reproduction leading to the rapid increase in the population soon after. Experience in culturing Diatoms have shown that a fresh change of the culture medium with addition of soil extract or sea-weed extract promotes rapid growth. A comparable favourable change appears to take place in the sea also on the west coast of India during this season, when owing to strong wind action, the water and sea bottom are well agitated thus bringing about dissolution into the water of the locked-up nutrients and other essential growth-promoting substances. The sea-water at this time is more or less an ideal culture medium. This state obtains for some time. Later, the intensity of the phytoplankton bloom subsides though apparently there is no deficiency of any of the obvious nutrients. It seems very probable that some substance, organic or inorganic, having got exhausted, acts as a limiting factor. That this is present in the

bottom sediment is indicated by the fact that there is a blooming of the phytoplankton more or less invariably following a period of strong winds which help mixing up of the water layers and bottom sediment.

The decline in the standing crop in the following season, north-east monsoon, could also be due to the *senescense* of some of the elements following a period of rapid multiplication, for, such a state has been observed in cultures of Diatoms even when the medium contained all nutrients; reference to this state has also been made by some authors (Marshall and Orr, 1930; Harvey *et al.*, 1935; Riley, 1943; Gross and Koczy, 1946; Iyengar and Subrahmanyan, 1944; and Subrahmanyan, 1947). Riley (1952, p. 63) states: " It is apparent from the experiments that the seasonal cycle is partly controlled by biological or chemical factors. Growth rates were high during the time when the spring flowering was developing; later they became much lower. The change cannot be accounted for by any of the observed environmental factors. However, the existence of reduced growth rates in the experiments subequent to the flowering is sufficient evidence that the population was kept at a low level by physiological limitation as well as by physical dispersal."

Therefore, it would appear that a phytoplankton bloom could occur only if the organisms concerned are also in their proper physiological state and the medium at that time is in a suitable composition. Such a condition appears to be fulfilled, ideally, during the south-west monsoon season on the west coast.

The magnitude of the standing crop is also affected by the zooplankton and nekton organisms present in the water which graze on the plant crop and are dependent on it for their existence. During the south-west monsoon season, the standing crop of phytoplankton is at its peak and though the grazers, zooplankton, also are on the increase and even attain a peak in numbers during the same season, the rate of production of phytoplankton appears to be so high as to maintain a high bulk in the water and exceed that of the animal population. During the following season, the standing crop is of a lower magnitude. During this season, herbivores in the form of Copepods and other zooplankters also occur in abundance as also plankton feeding fishes so much so the crop appears to be heavily grazed down. It is also possible that the rate of production of phytoplankton also slows down for reasons indicated earlier thus leading to a lower order in the magnitude of the crop as compared with the earlier period. Details of the relationship between phyto- and zooplankton and nekton have been discussed elsewhere (Subrahmanyan, Part I).

VIII. PHOSPHATE CONTENT OF THE WATER AND FISHERIES

In the English Channel, Russel (1935 *b*, 1936 *b*) has observed a parallel relationship between the quantity of phosphate in the water at the beginning of the year and the abundance of young fish. " This winter maximum is a measure of the amount of phosphate available for the following season's crop of phytoplankton and indirectly may control the survival of the larval planktonic stages of many marine animals " (Cooper, 1938 *c*, p. 183). An impoverishment of the phosphate in the water, coincident with a change in the type of plankton—which also distinguishes a different water mass—(Russel, 1936 *a*, *b*, *c*, *d*; Wilson, 1951; Wilson and Armstrong, 1952, 1954) is reflected in the fall in the abundance of fish. Thus, it was noticed that the thirties were poor years compared with the twenties when the phosphate content was higher (Cooper, 1938 *c*; Russel, 1935 *b*, 1936 *b*). To quote Harvey (1945, pp. 152–53), the changes in the phosphate content " indicates the water's biological future.... The relation, found by Russel between the fertility of the area and the winter store of phosphorus in the water, is a definite step" towards gauging "the extent of fishing which would give maximum return for human effort without unduly depleting the stock". Cooper's (1948 *c*) study of the relationship between phosphate content of the water and fisheries show that the commercial landings of some fishes follow major changes in the nutrient content of the overlying water with a time-lag of some years. The dependence of the bottom in-fauna upon the fertility of the overlying water is also indicated.

Naturally, the above cited accounts led to an examination of the phosphate content of the water recorded here on the west coast of India and the fish landings. Though the period of investigation has not been of sufficiently long duration as in the English Channel, the data relating to the fish landings for about 5 years appear to indicate a parallel relationship between the phosphate maximum recorded during the year and also the integral mean phosphate content of the water of the years. The time lag is bound to be shorter in this peculiar tropical environment where the nutrient cycles and metabolism of the organisms are different. The data are shown in Table V. While the parallel relationship is seen reflected in the fluctuation of the total fish landed, the mackerel and residual landings, the oil-sardine shows a somewhat opposite relationship; the quantity tends to increase with a fall in the phosphate content, probably because the fish is predominantly a phytoplankton feeder and as an inverse ratio obtains between phytoplankton production and phosphate content of the water. However, the clear-cut inverse relationship between phytoplankton and phosphate noticed in the temperate regions is

TABLE V

Phosphate and fisheries

Years May–April	Phosphate Mean integral value µg. at./L	Phosphate Maximum µg. at./L	Total landings Metric tons	Mackerel Metric tons	Oil-sardine Metric tons	Remaining fish Metric tons	Rainfall Inches
1949–50 ..	0·798	1·235	..	..	..	..	130·94
1950–51 ..	0·840	1·382	156,810	57,335	12,779	85,696	131·78
1951–52 ..	0·589	0·905	144,833	76,930	18,548	49,345	102·66
1952–53 ..	0·537	1·110	62,302	16,725	22,218	23,359	95·56
1953–54 ..	0·559	0·735	50,754	5,934	30,397	14,423	97·30
1954–55 ..	0·908	1·895	..	..	..	..	143·80

not emphasized on the west coast of India, though on some occasions such a one is seen on a sample-to-sample basis. In the present instance, the overall relationship is parallel; the phosphate attains the maximum during or immediately following the phytoplankton maxima and this complicates the picture of relationship between different groups of organisms. Nevertheless, it is felt that data gathered over several years, as for the English Channel, might reveal a relationship on a time-lag basis in our waters also, and when more knowledge accrues on the biology of the fishes constituting the fishery, it should become possible to predict much ahead the prospects of fish landings. For the present, the relationship observed on the west coast of India to those recorded in the English Channel is worth noting.

Again, another relationship becomes evident which is interesting. It was mentioned earlier that the freshwater influx into the sea during the south-west monsoon months might contribute a little phosphate to the nutrient content of the water, though not in significant quantities. Some evidence for this could be inferred in the somewhat parallel relationship seen between the integral mean value of the phosphate content and the total amount of rainfall during the years (Table V). The intensity of rainfall will depend on certain weather conditions and the same conditions might lead to a mixing up of the sea-water layers and the bottom sediment bringing about an increase in the phosphate content. It is also possible that the increased strength of the monsoon as indexed by rainfall might affect the conditions in the water in such manner as to lead to an increase in the dissolution of the bottom phosphate. Be that as it may, we are here concerned with the whole phosphate content in the water. The relationship pointed out between phosphate content and fisheries would, therefore, indicate indirectly a relationship with the rainfall, it being the same as with phosphate, a direct one. It may be interesting to mention in this connexion that Kalle (1949) has demonstrated a parallel relationship between precipitation and herring catches in the northern waters.

IX. SUMMARY

1. In this account, particulars relating to the environmental factors influencing phytoplankton production, such as temperature, salinity, phosphate, nitrate, silicate, and oxygen content of the sea-water are dealt with and compared with data from other regions in India and elsewhere, and their cycles and rôle are discussed.

2. There is a bimodal oscillation in the temperature of the water. The fall in temperature during the south-west monsoon season reaches optimum levels favourable for the growth of phytoplankton.

3. Salinity also shows a bimodal oscillation during the year, the lowest values being recorded during the south-west monsoon months, which, however, does not go beyond 4‰ of the highest values recorded. Changes in the salinity are found to have an important rôle in the metabolism of the Diatom cells when they are in a particular physiological state. A fall in salinity within limits acts as a stimulus to sexual reproduction of several species and their subsequent rapid vegetative multiplication leading to the peak bloom of the year. This aspect is discussed with reference to culture work on the Diatoms.

4. The nutrient salts, phosphates, nitrates and silicates show a seasonal fluctuation, the peaks in their concentration being attained during the south-west monsoon months, at the same time as or immediately following that of the peak phytoplankton bloom. It was found that there is always an abundant supply of these nutrients in the water for the growth of phytoplankton. The results are compared with earlier records from elsewhere and the source and cycle of the nutrients are discussed.

5. The dissolved oxygen content is never steady and oscillates very much. The peak in the oxygen-content is reached after the wane of the phytoplankton bloom. The relationships between oxygen-content, silicate metabolism and phytoplankton production are pointed out and discussed.

6. It was found that here also, as in the temperate waters, the ratio of 1 : 15 : 20 obtains between phosphate-P, nitrate-N and silicate-Si respectively in the waters which is very significant. Previous workers in this country were unable to establish any such relationship.

7. It is shown that the bottom mud on this coast is a rich storehouse of nutrients which are released into the waters during the south-west monsoon season generally and during periods of high winds in particular, when a mixing up of the layers occurs as also the bottom sediment. It is indicated that the mud also contains, besides inorganic nutrients, important organic substances and growth-promoters which are essential for the growth of phytoplankton, and the absence of which could act as a limiting factor.

8. Aspects of river influx contributing to the fertility of the inshore waters are discussed.

9. Besides the influence of external factors, the nature of the internal factors responsible for the multiplication of the Diatoms, the predominant element in the flora, is pointed out and its relationship with the environment is discussed.

10. It was found that a parallel relationship exists between both the phosphate maximum recorded during the year and the integral mean phosphate value of the years on the one hand and total fish landed on the other.

11. A somewhat parallel relationship also exists between the integral mean phosphate values and the total annual rainfall during the years; therefore, fish landings show indirectly a parallel relationship, similar to phosphate, with the annual rainfall.

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STUDIES IN THE INDIAN AQUATIC FUNGI

III. Sexual Reproduction in *Gonapodya polymorpha* Thaxter

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Received June 4, 1958

(Communicated by Dr. S. N. Das-Gupta, F.A.SC.)

In an earlier paper of the series[2] a description has been given of *Gonapodya polymorpha* and *G. prolifera* growing on fruits. Since then these fungi have been collected a number of times from different ponds and ditches in the neighbourhood of Lucknow. Members of the Monoblepharidales are considered to grow at a temperature of 8–11° C. but both the species of *Gonapodya* were found thriving well during summer in this country when it becomes extremely hot.

In 1953 some fruits of guava baited in one of the tanks in the departmental garden for capturing aquatic fungi were found to be covered with tufts of delicate, pale-grey flexuous hyphæ. Closer examination revealed that these hyphæ belong to *G. polymorpha*. The fruits on which the fungus was growing was brought in to the laboratory and a few of them were preserved in picroformol. The remaining ones were washed under running tap-water and then each fruit was put into a beaker filled with sterile distilled water. A few of these were kept in an oven at a temperature of 37° C. while the rest were allowed to remain in the room temperature. A little of the material from these cultures was examined at definite intervals and the water was changed every two or three days when bacteria appeared as a film on the surface. By this method the cultures were kept going for a long time. Attempts were made to grow this fungus on boiled split hemp seeds but so far it has not been successful.

It was found that the fruits kept at a temperature of 37° C. favoured the growth of *Gonapodya* for a few hours but after that bacteria developed rapidly, destroying the fungus completely in a short time. The fruits kept at room temperature continued to show the growth of the fungus for a number of days. On the seventh day while examining material kept at room temperature it was found that apart from the ordinary sporangia of *Gonapodya* there were others which were rounded in their shape and which contained

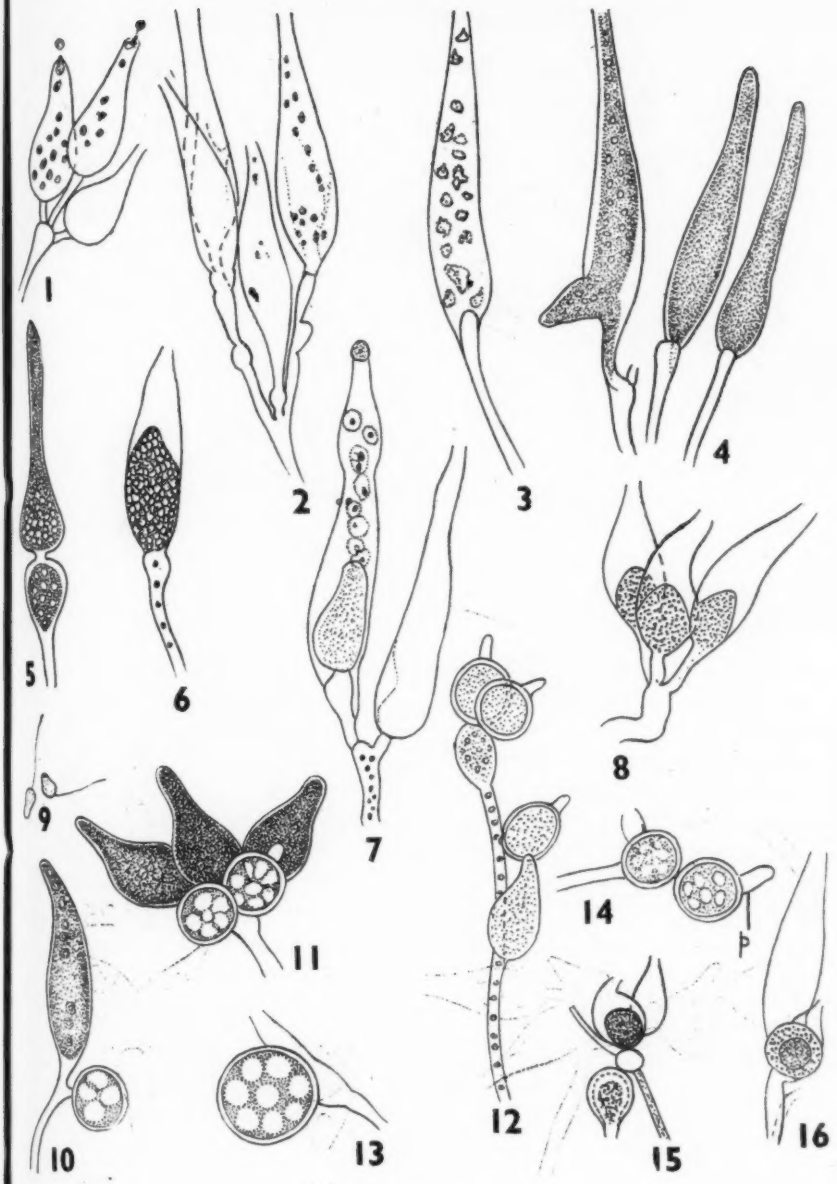
zoospores of bigger size than the ordinary sporangia. Careful examination of the cultures revealed that these were the female gametangia formed during the sexual reproduction of this fungus which is described in this paper. Since, in the earlier paper asexual reproduction was not described it is given below.

ASEXUAL REPRODUCTION

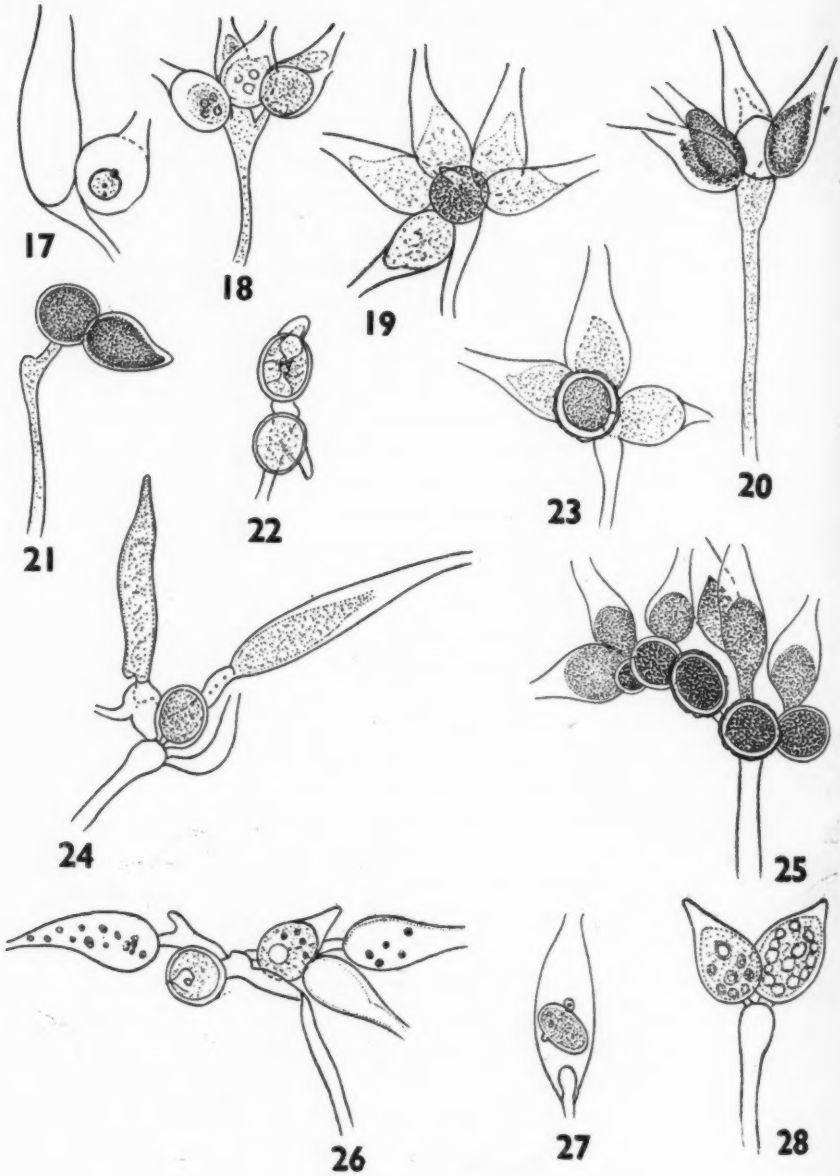
The sporangia of this fungus may be terminal and solitary or borne in clusters. They are variable in size and form but usually oval, 50–60 μ by 20–30 μ, tapering abruptly to form a blunt tip (Text-Fig. 1). Some of them may be long measuring 120 by 25 μ (Text-Figs. 2, 3 and 4). The contents of the sporangia in the young stage are homogeneous and highly refractive. As the sporangia mature they are cut off from the hyphæ by a cross-wall and cleavage furrows divide the contents into a large number of zoospore initials (Text-Fig. 6). The maturation of the zoospores take place completely inside the sporangia by the deliquescence of the apex of which a circular pore is formed. The zoospore creep in amoeboid fashion through this pore becoming elongated as they emerge and assuming a spherical shape outside the sporangia (Text-Fig. 7). The zoospore remains attached to the mouth of the sporangium by its flagellum for a short period and in the meantime other zoospores continue to emerge. Three or four of them may thus remain attached to the mouth of the sporangium but ultimately they jerk themselves free and dart away. At this stage the long flagellum can be seen trailing behind the zoospore which moves in a gliding manner. In the meantime considerable swarming of the spores takes place inside the sporangium and they seem to be eager to reach the outside. The last one or two zoospores may fail to emerge and may either disintegrate or germinate and produce a germ tube inside the sporangium. Discharged sporangia usually proliferate and produce a second lot of zoospores (Text-Fig. 8). The zoospores are cylindrical in shape with a narrow apex from which protrude a number of small refractive granules. To the posterior end is attached a single long flagellum (Text-Fig. 9).

SEXUAL REPRODUCTION

Male and female gametangia are borne on the branches of the thallus either terminally or in clusters (Text-Figs. 10 and 11). The male gametangia are oval with an elongated apex, 50–55 μ by 22–25 μ while the female gametangia are spherical or subspherical (Text-Fig. 11 and Pl. III, Figs. 1–4). 16–24 male gametes are formed in each male gametangium. They are posteriorly uniflagellate like the zoospores and are discharged in the same manner. The female gametes are bigger in size and only 12–16 are formed in one gametangium (Text-Fig. 13). A beak-like papilla is formed at the distal



TEXT-FIGS. 1–16



TEXT-FIGS. 17–28

end of the female gametangium through which the gametes are discharged (Text-Fig. 14, *p*). Some of the female gametes are not discharged from the sporangium. In such cases they remain inside the sporangia and develop into oospores after fertilisation (Text-Figs. 15, 16 and 18). Usually one female gametangium has one discharge papilla but in a few cases two papillæ have been seen on the same gametangium (Text-Fig. 15). Both male and female gametangia are capable of renewal by internal proliferation. It appears that the male gamete fuses with the female gamete after they have been discharged. In some cases where the female gametes fail to be discharged the fusion takes place inside the female gametangium. Text-Figure 17 shows one such female gamete with part of the antherozoid still visible on top. Text-Figure 27 shows another undischarged female gamete to which are attached two antherozoids. After this fusion the zygote develops into a spherical, thick-walled resting spore. Many young resting spores were found scattered among the hyphæ and a few were seen inside the female gametangium. The latter ones when mature fill the gametangia measuring 15–20 μ and having a dark brown colour. The outer wall in the mature resting spores does not seem to be absolutely smooth. Text-Figure 23 shows a resting spore with a slightly warty outer wall. Germination of these resting spores was not observed.

The sexual process in *Gonapodya* was first observed by Cornu[1] in 1877 in the species, *G. prolifera*. *G. polymorpha* was unknown at that time. No other worker [3, 5, 6] had observed this process in the genus *Gonapodya* till recently when in 1954 Johns and Benjamin[4] published an account of the sexual reproduction in both *G. prolifera* and *G. polymorpha*. The stages in the sexual reproduction of *Gonapodya* given in this paper conform mostly to the one given by Johns and Benjamin.[4] It was found, however, that in the present case oospores were commonly but not always formed inside the female gametangia filling them completely. Young oospores are smooth-walled but when mature they are brown in colour and seem to have slight ridges on the outer wall. Johns and Benjamin[4] have described the oospores to be smooth-walled.

The development and reproduction of *Gonapodya* are being further investigated in this laboratory.

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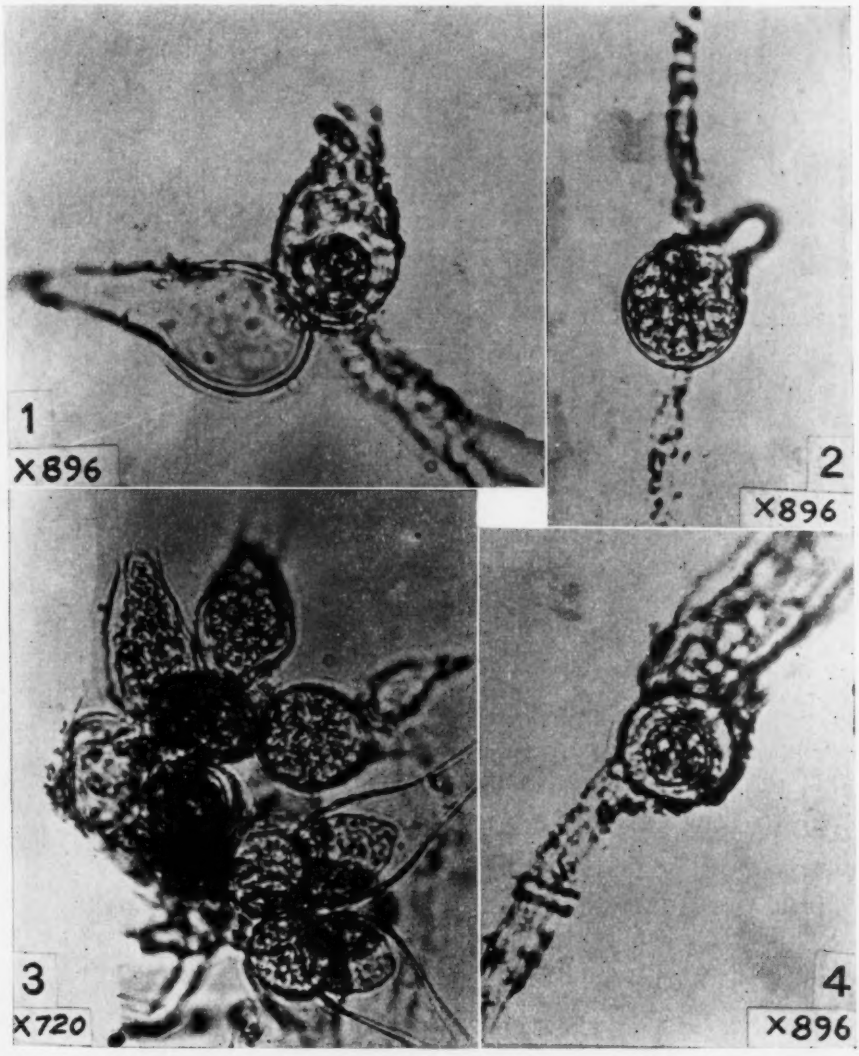
EXPLANATION OF TEXT-FIGURES

TEXT-FIGS. 1–16. *Gonapodya polymorpha*. Figs. 1–5. Sporangia of *G. polymorpha* showing different shapes, ×225. Fig. 6. Cleavage in a proliferating sporangium, ×225. Fig. 7. Emergence of zoospores from the sporangia, ×225. Fig. 8. Proliferation of sporangia, ×225. Fig. 9. Zoospores, ×225. Fig. 10. One male and one female gametangia, ×225. Female gametangium shows cleavage before forming female gametes, ×225. Fig. 11. Cluster of male and female gametangia, the latter showing cleavage and also the terminal papilla, ×225. Fig. 12. Female gametangia one of which contains a developing oospore. Fig. 13. Magnified view of female gametangium showing cleavage, ×325. Fig. 14. Female gametangia, one showing zoospores and the papilla, *p*, the other showing a developing oospore inside, ×225. Figs. 15 and 16. Female gametangia showing developing resting spores inside, ×225.

TEXT-FIGS. 17–28. *Gonapodya polymorpha*. Fig. 17. The female gametangium showing one female gamete inside with part of the male gamete visible on top, ×225. Fig. 18. A goup of female gametangia, ×225. Fig. 19. On female gametangium among 4 male gametangia, ×225. Fig. 20. A group of male gametangia, ×225. Fig. 21. One female and one male gametangia. Fig. 22. Female gametangia showing beginning of cleavage, ×225. Fig. 23. Three male gametangia with one female gametangium in the centre containing a fully enveloped oospore with slightly warty wall. ×225. Fig. 24. Two zoosporangia and one female gametangium in which oospore is developing, ×225. Fig. 25. A group of male and female gametangia. Two of the latter contain mature oospores with slightly warty walls, ×225. Fig. 26. Male and female gametangia, ×225. Fig. 27. Female gametangium with one egg showing fusion of male and female gametes, ×225. Fig. 28. Male gametangia, ×225.



FIGS. 1–4. *Gonapodya polymorpha*. Fig. 1. A female gametangium showing an egg inside, which is developing into the oospore, ×896. Fig. 2. A single female gametangium with discharge papilla, ×896. Fig. 3. A group of male and female gametangia. Some of the latter have developing resting spores inside, ×720. Fig. 4. A female gametangium showing a developing resting spore inside.

STUDIES IN THE INDIAN AQUATIC FUNGI

IV. *Catenaria sphærocarpa* on *Spirogyra* Sp.

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The genus *Catenaria* was established in 1876 by Sorokin[10] for a fungus which he found parasitic in eel-worms. He named it *C. Anguillulæ* which caused an epidemic in the eel-worms and eventually killed most of them. Sorokin described *Catenaria* to be made of septate mycelium in which the segments swelled up to form sporangia. At maturity, therefore, the fungus consisted of a chain of sporangia separated by short isthmuses. The sporangia liberated their zoospores by means of a discharge tube. Sorokin found this fungus to infect also eggs of aquatic animals such as rotifers.

In 1885 Dangeard[7] found the same fungus on eel-worms. He found that *Chytridium zootocum* described by A. Braun in 1856 was nothing but *Catenaria Anguillulæ*. He also found that the same fungus was described and figured by Villot in 1874 before Sorokin had found it. Villot had mistaken it to be a freshwater alga which he found parasitising hair worms of the genus *Gordius*. Dangeard was able to culture this fungus in *Nitella tenuissima* by placing the alga in vessels containing infected eel-worms. In the Nitella cell he was able to see the fungus much more clearly and he found it to be dichotomously branched. He was the first person to note the existence of rhizoids which arose both from the sporangia and from the isthmuses. He has given a description of the sporangia and the zoospores.

In 1901 Constantineanu[5] found in eel-worms *C. Anguillulæ*, but both the sporangia and the discharge tube in his material were larger than those described by Sorokin.

Serbinov had described *C. pygmæ* in 1907 which was considered doubtful by von Minden[12] in *Kryptogamen flora der Mark Brandenburg*, because Serbinov's fungus had only a single sporangium arising from a highly developed rhizoidal system whereas *Catenaria* is distinguished by the chain of sporangia and the narrow isthmuses.

In 1927 Butler, J. B. and Buckley[3] found *C. Anguillulæ* on the eggs of *Fasciola hepatica*, the sheep liver-fluke in Ireland.

In 1928, Butler, E. J.[2] found the same fungus occurring as a parasite in the eggs of the liver-fluke *Fasciola hepatica*. His fungus also had larger measurements than described by others. He has given a description of the sporangia, zoospores and the method of discharge.

In 1929 Buckley, J. J. C. and Clapham[1] found *Catenaria* parasitising Helminth eggs.

In 1932 Butler, J. B. and Humphries[4] grew this fungus for the first time in artificial culture. The best medium used by them was equal parts of a 0·25% solution of agar in water, concentrated fluke ova extract in water and water.

In 1934 Karling, J. S.[8] isolated from the roots of *Panicum variegatum* a *Catenaria* which was saprophytic and capable of growing on a variety of dead tissues. He found the thallus to be mono- or polycentric, the latter being predominant. In some there was no trace of any rhizoid whereas mostly the rhizoids were clearly seen. In insect eggs due to lack of space the fungus became condensed and crowded whereas in cells of Chara and Nitella it became mycelium-like and extended over a considerable length. Karling found the rhizoids to be separated from the thallus at maturity by cross walls. The zoosporangia were extremely variable in size and shape. Sometimes enlargements occurred in the thallus resembling " sammelzellen " of *Physoderma* and *Cladochytrium*. Zoospores were produced in abundance and were mostly uniciliated although a few biciliated and fewer triciliated ones were also found. In two cases he observed fusion between zoospores.

In 1938 Karling[9] published a further study of *Catenaria* in which he named this saprophytic species as *C. sphærocarpa* sp. It was found in dead algal cells and was cultured in cooked root tips of *Zea mays* and *Allium cepa*. The zoospores of this species were distinguished from those of *C. Anguillulæ* in being large and spherical with a single conspicuous refractive globule and a characteristic type of swimming. The zoosporangia were predominantly spherical and brown resting spores were found.

In 1945 Couch[6] isolated from soil a species of *Catenaria* growing as an obligate parasite on species of *Allomyces* and *Blastocladiella simplex*. He described the different stages of infection on *A. anomalus*, development of the thallus, zoosporangia and resting bodies of the fusion of gametes. He named this fungus *Catenaria Allomycis*. He also isolated *C. Anguillulæ* from soil and grew it on a variety of agars in pure culture for the first time. He found that

it could be grown also on nematodes and grass leaves and that it could infect liver-fluke eggs. Zoosporangia were plentiful whereas resting bodies were rare. The resting bodies were found to germinate like those of *Blastocladiella*. The zoospores of *Catenaria* were found to be similar to those of Blastocladiales with nuclear cap, side body and numerous fat bodies. The method of discharge, swimming and germination of the zoospores were typical of the Blastocladiales. The resting body was formed in a zoosporangial case as in the Blastocladiales. Septations were found to be irregular as in *Allomyces*. Life-cycle of *C. Anguillulæ* was parallel to that of *A. anomalus* or *Blastocladiella simplex* while that of *C. Allomycis* was parallel to that of *A. cystogenus*. On the basis of these discoveries Couch transferred *Catenaria* from the Chytridiales to the Blastocladiales in 1945. Subfamily Catenarioideæ of Sparrow was raised to a family Catenariaceæ fam. nov.

From India no report of the fungus has so far been made. In November 1953 a collection of *Spirogyra* from Sultanpur Road showed the presence of a species of *Catenaria* inside the cells. Part of the collection was immediately preserved in Picroformol for further study. Fresh healthy *Spirogyra* from nearby tanks and ponds were collected and attempts were made to infect them by placing a few infected filaments along with the healthy ones. Although these were kept for a number of days inside the laboratory no infection took place. A number of hanging drop cultures were made from the filaments containing the fungus in order to study the development, the mode of escape and the nature of the zoospores. Although in some cases the zoospores were swarming inside the sporangia none of them were found to be liberated. Perhaps due to the change in the environment the sporangia failed to open out and allow the zoospores to escape.

A number of slides showing different stages of development were made from fresh as well as preserved material. In making these slides a few infected filaments were put in a drop of 70% glycerine on a slide and after teasing them out a coverslip was placed on top. The glycerine was then allowed to concentrate and the slide was sealed with a proper seal. Slides showing different stages were studied under the microscope to find out the life-history of the fungus.

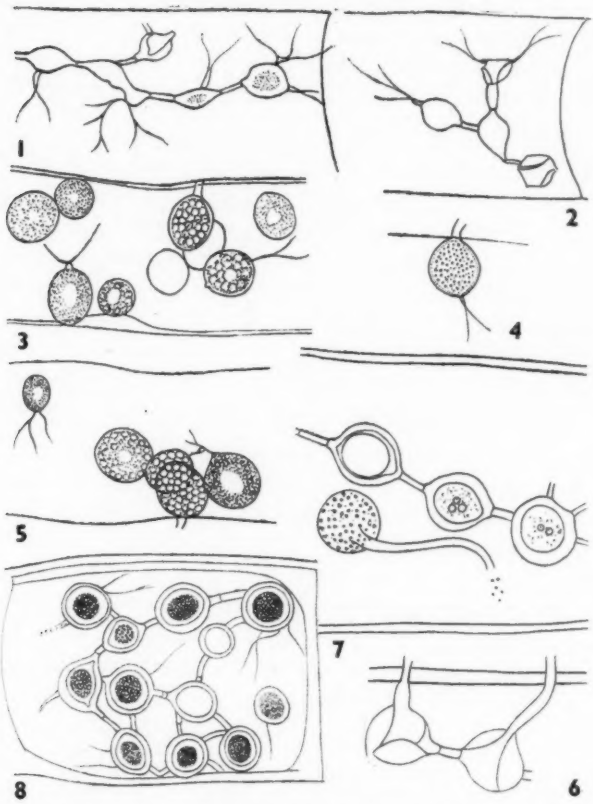
The fungus occurred mostly in disintegrated cells and sometimes in partly green cells of *Spirogyra*. From experimental studies and the observation of the form under natural conditions it appears that the fungus is a saprophyte as described by Karling[8] for his species. The presence of the fungus in the partly green filaments perhaps indicates that the cell was no longer in a living condition but the green colour had not completely disappeared. Karling

has found that this fungus can be grown on a wide variety of plant and animal tissues the most favourable one being dead onion roots. No attempts were made by the author to culture the fungus on dead tissues as at the time of collection the presence of the fungus in partly green cells gave the impression that it might be a parasite and hence only healthy algal filaments were used as baits. Moreover in this collection the fungus had already started formation of resting spores. In fact resting spores were plentiful while sporangia were found only in very few cells.

DESCRIPTION

The thallus exhibits a great range of variation in size and shape. The cells of the thallus may be spherical, elongated or irregular sometimes showing the presence of rhizoids and sometimes completely lacking in rhizoids. Usually the thallus is polycentric and the cells of the thallus are spherical. In Text-Fig. 1 is shown a thallus in the early stages of development. The different cells of one thallus are connected by narrow isthmuses. In the very early stages of development there are no septa and the whole thallus is continuous but at maturity of the sporangia the contents from the connecting parts also migrate into the sporangia and septa are formed separating one sporangium from the other which are then connected only by the narrow sterile isthmuses (Text-Fig. 6). Rhizoids arise both from the surface of the sporangia and from the isthmuses as shown in Text-Figs. 1 and 2. They are thick to start with but as they branch and rebranch they become narrower and at the extremities they extend as fine tapering filaments. The rhizoids are separated from the sporangia by cross walls. Species of *Catenaria* show enlargements on the rhizomycelium which are known as " sammelzellen " formation but the host tissue in the present case was not favourable to demonstrate this phenomenon. According to Karling, *Chara* and *Nitella* are the best hosts for the study of " sammelzellen " formation.

The young sporangia are filled with granular contents (Text-Fig. 3). As they mature cleavage furrows are formed which mark out the zoospores (Text-Figs. 4 and 5). At maturity the sporangia are separated from each other by narrow isthmuses as stated before. In shape the sporangia are mostly spherical (Text-Fig. 9) although various other shapes are not uncommon. In size they vary from 15–40 μ by 8–30 μ. Each sporangium produces one exit tube which tapers from the base to the apex. The exit tubes are of varying length, some being considerably long and others rather short and in both cases they may be straight, curved or irregular, 10–40 μ by 5–8 μ. The exit tubes may end at the surface of the host cell-wall or may project beyond it (Text-Figs. 3 and 6). At the point where they pass through the host cell-wall



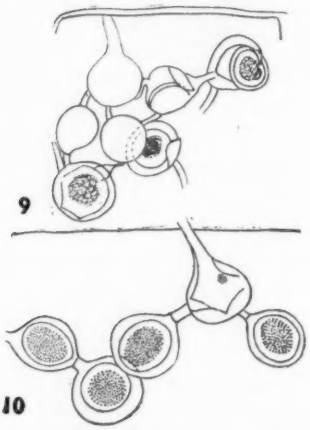
TEXT-FIGS. 1–8. *Catenaria sphærocarpa* in *Spirogyra*. Figs. 1–2. Thallus in the early stages of development showing young sporangia and rhizoids, ×150. Fig. 3. Young and maturing sporangia, ×217. Fig. 4. A single sporangium, ×217. Fig. 5. Sporangia with zoospores, ×217. Fig. 6. Old collapsed sporangia showing exit tubes and connecting isthmuses, ×200. Fig. 7. Resting spores and one sporangium with a long exit tube containing zoospores. Fig. 8. Resting spores of the fungus inside a cell of *Spirogyra*.

they are reported to be rather constricted especially in *C. Anguillulæ* but according to Karling[8] this depends on the state of degeneration of the host tissue. In this case no constrictions were found.

The zoospores are formed inside the sporangium and when they are mature they are liberated through the exit tubes. Text-Figure 7 shows one mature sporangium with a long curved exit tube and maturing zoospores inside. As stated before considerable swarming of the zoospores have been noted inside the sporangia but their liberation has not been

observed. Karling[8] has stated that if due to environmental changes there is delay in dehiscence of the tip of the sporangial tube then such swarming takes place inside the sporangium. The usual method of liberation of zoospores, according to him, is that they escape to the outside one by one. After the discharge of the zoospores the sporangia collapse as seen in Text-Fig. 6.

Resting spores of the fungus were plentiful. Almost all the cells contained some resting spores. They are large and spherical, 20–30 μ by 25–40 μ, with thick heavy brown-walls and granular contents as seen in Text-Figs. 7 and 10. They too are joined to each other by short narrow isthmuses. Karling[8–9] has found resting spores of this species only on two occasions in the cells of *Nitella* and *Chara* and he considers them to be just ordinary sporangia which have encysted and developed thick walls. Connected with the resting spores are found ordinary zoosporangia which have discharged their contents and collapsed as shown in Text-Fig. 10. This fact may also indicate that the resting spores are nothing but encysted zoosporangia.



TEXT-FIGS. 9 and 10. *Catenaria sphærocarpa*. Fig. 9. Old sporangia and mature resting pores of *Catenaria* inside the cell of *Spirogyra*, ×300. Fig. 10. Resting spores and one old collapsed sporangium of the fungus inside the host cell, ×400.

The germination and further development of the resting spores of this fungus have not been observed by the author. Karling also has not been able to follow the germination but in the case of *C. Allomycis* Couch has followed the development of the resting spore completely. According to him when favourable conditions are available the resting spore absorbs water and swells thus causing its outer wall to crack. The inner wall then produces a long exit tube and this structure behaves like an ordinary zoosporangium liberating

its zoospores to the outside through the exit tube. In the present case there are indications to show that the resting spores develop like those of *C. Allomycis.*

Identity of the fungus.—At first sight the fungus almost looks like *Myzocytium* but closer examination shows the size and shape of the sporangia to be quite different. The short isthmuses connecting the sporangia and the rhizoids arising from both the sporangia and the isthmuses are also distinctive characteristics and there was no difficulty in identifying the fungus as a species of *Catenaria.* Of the three known species of *Catenaria* both *C. Anguillulæ* and *C. Allomycis* are parasites, the former in liver-fluke eggs and the latter in *Allomyces* and *Blastocladiella.* Only *C. sphærocarpa* has been found as saprophytic in green algæ. In the present case the fungus grows saprophytically in *Spirogyra.* As stated before it failed to infect healthy *Spirogyra* filaments placed in contact with it. Moreover this fungus has predominantly spherical zoosporangia and brown resting spores as distinguished from usually uteriform zoosporangia and hyaline resting spores of *C. Anguillulæ* as noted by Karling.[8] According to him the zoospores of *C. sphærocarpa* are large and spherical with a single conspicuous refractive globule and a characteristic type of swimming while those of *C. Anguillulæ* are oval with several small refractive granules. The zoospores of the present fungus agree with those of *C. sphærocarpa.* According to Couch the resting spores of *C. Anguillulæ* have light brown walls, hyaline contents and a characteristic pip on its surface. The resting spores of *C. Allomycis* are pale brown to pinkish, and globose, subglobose, ovoid, elliptic, pyriform or irregular in shape. The fungus described in this paper mostly conforms to the description of *C. sphærocarpa* and therefore it is here identified as *C. sphærocarpa.*

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THE METABOLISM OF *PLOTOSUS ANGUILLARIS* (BLOCH) IN VARIOUS CONCENTRATIONS OF SALT AND OXYGEN IN THE MEDIUM

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Introduction

A matter which is of interest in fisheries both from the industrial and scientific points of view is the ability of fish and other animals to migrate from sea to freshwater and *vice versa*. In spite of the attention which this field of research has claimed, much still remains to be explained. The nature of the adaptive processes that the individual undertakes is not fully known.

The relation between osmoregulation and metabolism with which this paper is concerned has been studied in a number of different animals by Sumner (1905), Schlieper (1929 and 1955), Beadle (1931), Keys (1931 and 1933), Raffy (1932 and 1933), Schwabe (1933), Krogh (1939), Wikgren (1953), Flemister and Flemister (1951), Lofts (1956), Gross (1957) and others. Of these one school believes that the increase in osmotic load is directly reflected in an increase in the metabolic rate. This is however denied by the other school which considers that the actual cost of osmotic regulation in terms of increased metabolic rate is relatively negligible. But whatever the position, it is universally believed that in the fish the gill is as important an organ of osmoregulation (excretion) as it is of respiration. As stated by Smith (1930 and 1953) the teleost can live in salt-water not because of any specialization of its kidney but because of the ability of its gills to retain the water and withdraw the salts from the blood and discharge them into the sea. This process according to the same author, requires, proportionate to the magnitude of work involved, valuable energy expenditure.

With the object of studying the problem further, a marine cat-fish: *Plotosus anguillaris* (Bloch) was used in the investigation. Even though no measurement of the osmotic changes of the blood of the fish was made, evidence is gathered from the study of the relationship of metabolism to changes

in salinity of the environment in order to demonstrate that the cost of regulation is indeed significant.

MATERIALS AND METHODS

Plotosus anguillaris (Bloch) occurs in this area from January to June in dense patches of some 300–500 individuals in a shoal. Each shoal is remarkably homogeneous regarding the size of the individuals constituting it. Probably the individuals of a shoal belong to a single brood. Fingerlings and adults of both sexes not actively breeding, and belonging to different shoals were employed in this work, and they weighed between 5 and 45 gm. Since none of the adult fish used was in the active breeding phase the effect of sex as noted by Keys (1931) in the killifish has been overlooked in this study. The fish on capture were held in 500 gal. cement tanks supplied continuously with sea-water. The average temperature in these holding tanks during the period of study was 29° C. and the experiments were also performed in that temperature. For obtaining salinities lower than that of sea-water the local tap-water of average salinity 0·4‰ and pH 7·5–8·0 was mixed with the former. The salinity in this case was maintained at any desired level by using a constant level mixer. For obtaining salinities higher than that of sea-water one lot of commercial sea-salt was used throughout, both for acclimation and experimental purposes. Following is the analysis of the salt used:—

Sodium chloride	97·77% M.F.B.
Calcium sulphate	1·04% M.F.B.
Magnesium sulphate	0·55% M.F.B.
Magnesium chloride	0·23% M.F.B.
Insolubles	0·84% M.F.B.
Moisture	7·84%

The water in the acclimation tanks for higher salinities was aerated throughout and the water itself renewed every week. The pH in these tanks ranged between 8·0 and 8·5. This procedure was adopted since it was not possible to maintain a continuous supply of high salinity water in the acclimation tanks. In all cases, however, the oxygen concentration was maintained at air saturation levels and the range of pH remained within 7·5–8·5.

The active metabolism and the standard metabolism were measured in the apparatus described by the author (Job, 1955 and 1957), but with the addition of a constant level mixer to the standard metabolism apparatus.

While the diluted sea-water cannot be compared with the concentrated 'sea-water' particularly with regard to the ionic composition the results presented are not without significance in that all the fish were subjected to the same conditions and their responses were studied (*cf.*, Quigley, 1928). Because of the difficulties involved and on the strength of Young's findings (Young, 1938), the use of commercial sea-salt was found to be the closest one could get to normal concentrated sea-water.

In the acclimation tanks the raising or lowering of the salinity was gradually done at the rate of 1–2·5‰ in 48 hours and maintained constant at the two other levels 40·0 and 12·5‰ besides sea-water, for not less than 20 days. The salinity values given in this work represent always the average values, the fluctuations being kept within 1·5‰ in any one level.

In the experiments the initial concentration of oxygen was brought to an average partial pressure (pO_2) of 150 mm. Hg by bubbling air or oxygen through air diffusers made from coral stones (*Millipora* sp.) suitably ground and adapted in the laboratory. By using groups of five fish at a time (average weight 7·26 gm.) some preliminary experiments were performed to estimate the tolerance range of salinity for this species. It was found that fish held in sea-water at 29° C. tolerated from 2–52‰ and that salinities on either side of this range proved lethal in 48 hours. In the same way preliminary experiments on the active oxygen consumption indicated that the level of oxygen at which the fish died (lethal level of oxygen) was lower when tested in salinities of 12·5 and 40·0‰ than in any other salinity including its own natural habitat salinity of 30·4‰. In order to examine whether these two other salinities had any significance, fish were acclimated to 12·5 and 40·0‰ and experiments performed with them as well as with those held in sea-water (S.W.) of 30·40‰ salinity.

Except in the experiments in distilled water no adjustment of the pH of the experimental medium was attempted. Quigley (1928) found that buffers used for this purpose had a toxic effect upon the fish. The active metabolism of S.W. fish was measured in test salinities of 0·0, 0·4, 12·5, 30·4, 44·2 and 60·0‰; and those of fish acclimated to 12·5‰ in test salinities of 0·4, 12·5, 30·4 and 44·2‰; and those acclimated to 40·0‰ in test salinities of 0·4, 12·5, 18·0, 40·0 and 60·0‰. The measurement of standard metabolism was however restricted to two salinities only where the acclimation and test salinities were the same and were respectively 12·5 and 30·4‰. In the experiments the initial and final pH were always measured and the range was between 8·5 and 7·5 respectively.

RESULTS

The data of oxygen consumption in relation to weight and under pO_2 ranging from 150–25 mm. Hg for the respective salinities were submitted to the same statistical treatment as earlier (Job, 1957) and are presented in a double logarithmic grid in Figs. 1, 2 and 3 for fish acclimated to salinities of 30·4, 40·0 and 12·5‰ respectively.

As the experiments were carried through to the point of death in the active metabolism chambers, the data collected on these lethal levels were also treated as above. These, together with the data on standard metabolism measured for fish of average weight 7·26 gm., will be presented and discussed later.

DISCUSSION

The basic data presented in Figs. 1–3 were used in obtaining the mean rates of oxygen consumption for the three weights 5, 15 and 45 gm., as this would facilitate comparison of the responses of the respective sizes to environmental stress. The main features of the experiments are: (1) The standard metabolism was measured at the two acclimation levels 12·5 and 30·4‰ with the oxygen concentration in the medium being maintained in the region of air saturation values during the entire period of the experiments; (2) the active metabolism was measured both in the three acclimation levels 12·5, 30·4 and 40·0‰ as well as in other test salinities, but with the oxygen tension in the medium falling from an average initial level of 150 mm. Hg to the lethal level.

The difference between the active metabolism in the acclimated salinities and that in the test salinities in any series is that in the former the falling oxygen tension in the medium is the only important extraneous factor; whereas in the latter the sudden impact of an entirely different salinity to which the fish was never exposed before, is an additional factor it must reckon with. Therefore, where the metabolism of fish is measured in the salinity to which they were already acclimated the term 'normal' metabolism is employed and where the acclimation and test salinities are not the same the metabolic response is termed 'instant' metabolism, purely as descriptive terms.

Active Metabolism, Size and Salinity: Data presented in the preceding three figures were used for obtaining the responses of a 5, 15 and 45 gm. fish to various salinities. Figure 4 illustrates the above, where the log. of oxygen consumption is shown against the salinity in a semi-logarithmic grid. Panel A shows the maximum rate of oxygen consumption when the pO_2

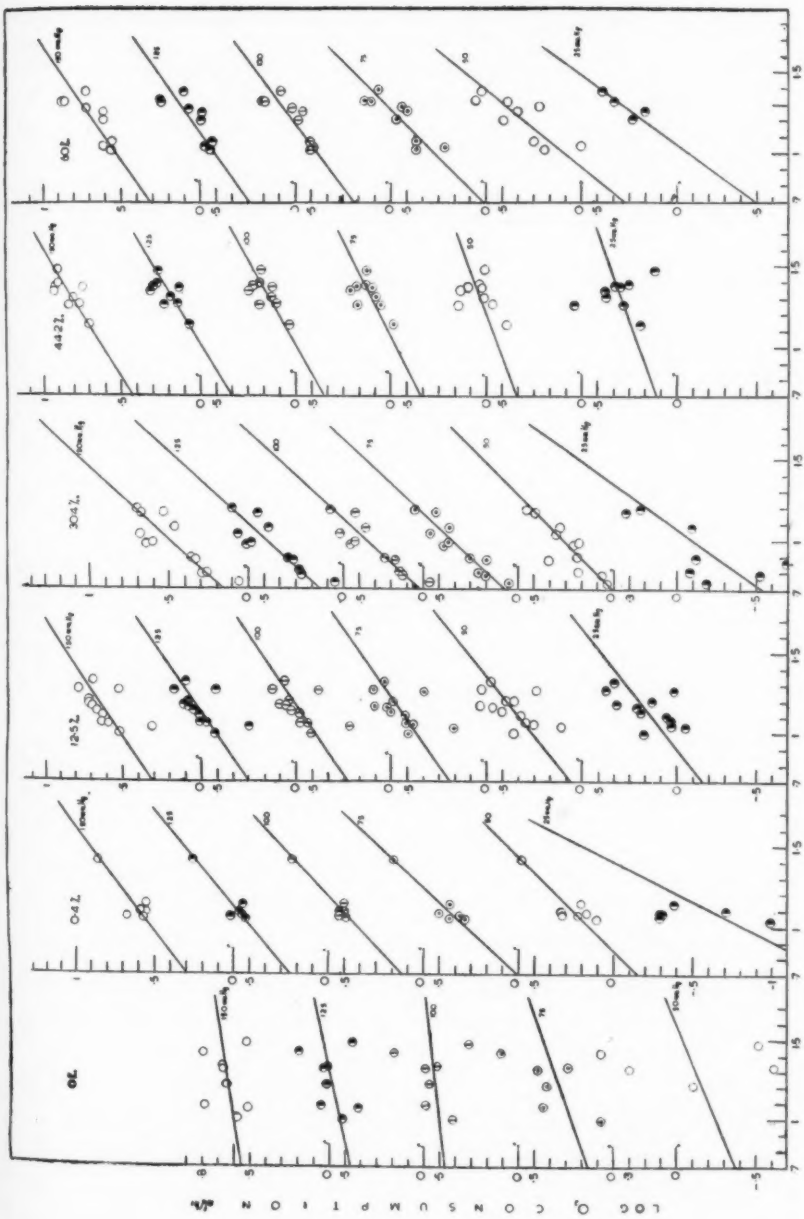

FIG. 1. Data of active metabolism at 29° C. of fish acclimated to that temperature and to sea-water salinity of 30·4‰. The partial pressures of oxygen are indicated at the upper end of each line. The test salinities are indicated in the respective compartments at the top. The lines of best fit were drawn according to the principle of least squares. The arrows along the ordinate indicate, in this double logarithmic plot, the corresponding cycle of the line immediately opposite.

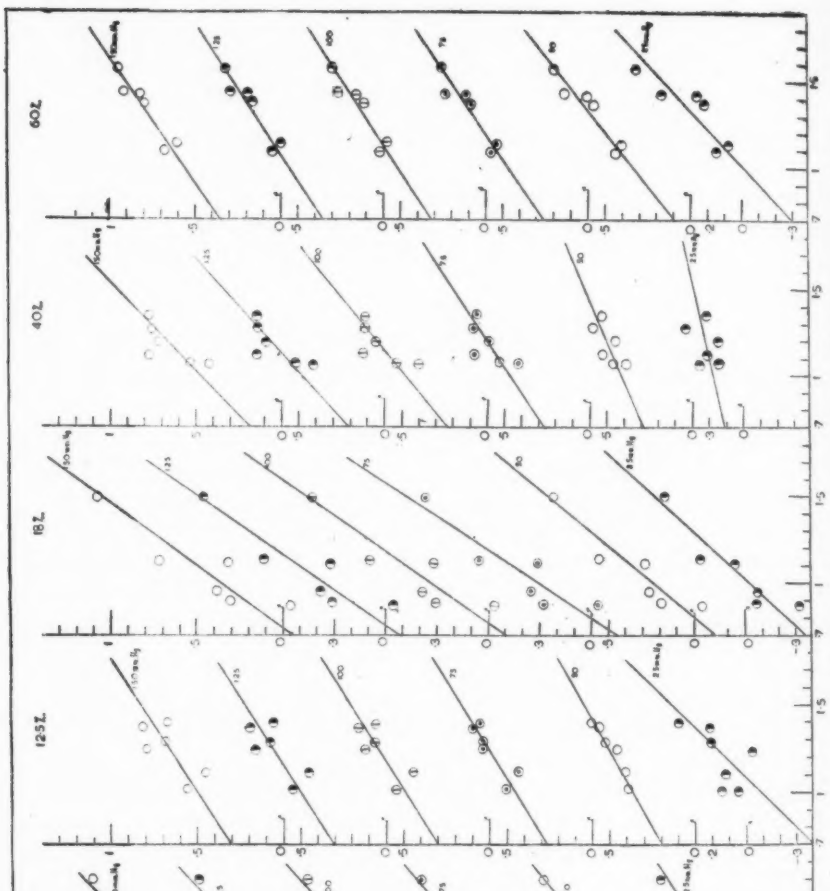

FIG. 2. Data as in Fig. 1, but for fish acclimated to 40·0‰ salinity.

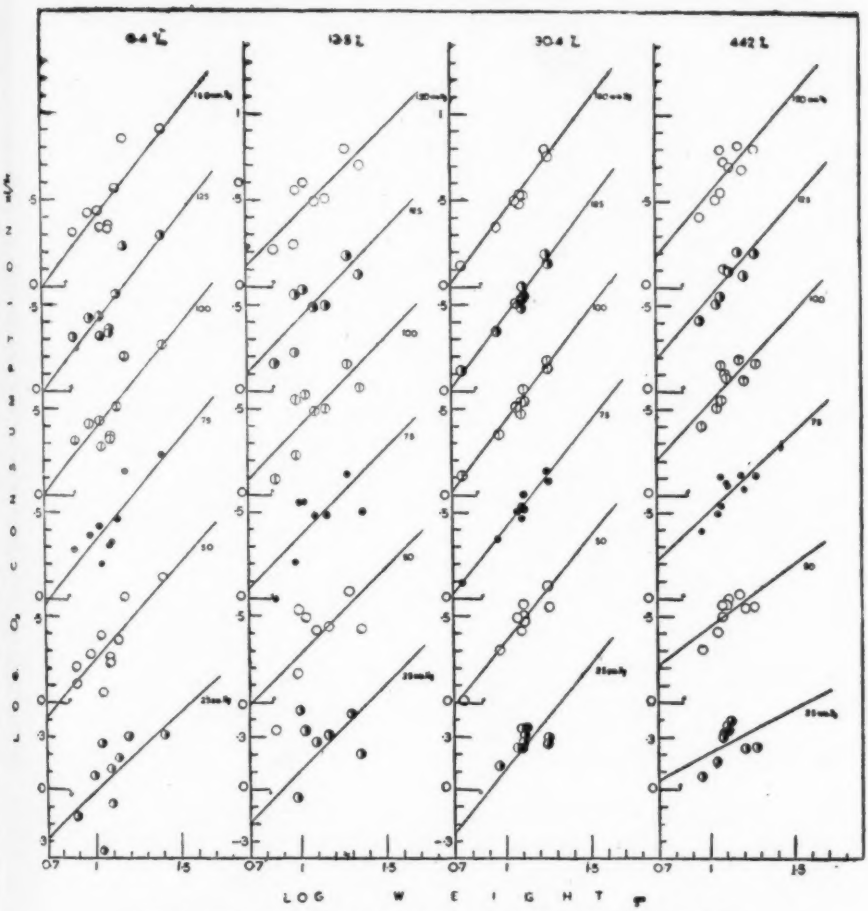

FIG. 3. Data as in Fig. 1, but for fish acclimated to 12·5‰ salinity.

is 25 mm. Hg in the medium and Panel B when it is 150 mm. Hg. The most striking feature of the figure is the variability of the responses particularly of the two extreme sizes which show almost opposite reactions, appearing to be mirror images of each other.

However in spite of these differences, within the respective weight groups the response of fish acclimated to sea-water and those acclimated to 12·5‰ are proportionately similar over the range of 0·4–44·2‰ salinity, and the curves follow the same pattern more or less. While the value of curve for

FIG. 2. Data as in Fig. 1, but for fish acclimated to 40·0‰ salinity.

the 5 gm. fish in 12·5‰ is lower than that for the same weight in S.W. acclimation, the comparison between the respective curves of the 45 gm. fish shows the opposite trend. But the difference is relatively small except beyond 35‰ when the low salinity curve diverges and becomes more widely separated. The curves for the fish acclimated to 40‰ follow the same course as those acclimated to S.W. except that the instant rates in 18‰ are such as to produce a marked fall or rise in the rates of the 5 and 45 gm. fish, thus distorting the curve in that region in relation to the others.

The curves in Panel A belong to the section that follows. But they are of interest here merely to indicate the trends in the uptake of oxygen as the pO_2 approaches the lethal point. Comparison of the curves in the two panels for the respective weights indicates that the 12·5‰ acclimated fish are least affected by the fall in the oxygen pressure probably because of the absence of other interfering factors besides pO_2. The greater fluctuations in the other two sets of curves could therefore indicate that as the fish approach the asphyxial levels of oxygen, the salinity of the medium reveals the extent of its interference with the uptake of oxygen. Even in this aspect the intrinsic differences between the 5 and 45 gm. fish are such that in the latter the 30·4 and 40‰ acclimation curves show greater similarity in the trend than does the 12·5‰ curve for that weight with either of the two curves. In the 5 gm. fish the closeness of the respective curves to one another is however limited only to certain sections of the range of salinity tested. The actual values for the curves are given in Table I. From Table I it can be said that the different sizes of fish respond differently to changes in the salinity of the medium, with reference to their active metabolism. If this is not entirely a metabolic response brought about by osmotic regulation, it will surely be granted that changes in salinity do impose a significant load on the gills or respiration so that the ability to utilise oxygen is measurably affected, almost instantaneously. The above conclusion is proved further in the relation of size and oxygen consumption, where the pO_2 in the medium restricts its uptake by the fish, the subject of the following section.

Size, Oxygen Tension and Salinity: In Fig. 5 is illustrated the metabolism of the fish of the three sizes as the oxygen tension in the medium falls from 150–25 mm. Hg and in different salinities.

Fish acclimated to 30·4‰ (*S.W.*).—The normal rates of oxygen uptake of fish tested in this salinity showed that they were proportionately the same for all sizes under all partial pressures of the gas. In Fig. 5 A, this is clearly indicated by the broken line representing the curve of the 45 gm. fish reduced

TABLE I

Maximum uptake of oxygen (ml./hr.) by 5, 15 and 45 gm. fish acclimated to 30·4, 40·0 and 12·5‰ and under 25 and 150 mm. Hg pO_2 and in different test salinities. Figures in parenthesis are lethal oxygen levels (ml./lit.)

Test sal ‰	5 gm. Fish			15 gm. Fish			45 gm. Fish		
	Leth O_2 ml./lit.	O_2 uptake ml./hr. when pO_2 is		Leth O_2 ml./lit.	O_2 uptake ml./hr. when pO_2 is		Leth O_2 ml./lit.	O_2 uptake ml./hr. when pO_2 is	
		25 mm. Hg	150 mm. Hg		25 mm. Hg	150 mm. Hg		25 mm. Hg	150 mm. Hg
For fish acclimated to 30·4‰									
30·4	(0·70)	0·29	1·45	(0·51)	1·41	5·01	(0·38)	2·69	17·18
0·0	(3·60)	*0·42	3·63	(2·31)	*0·66	4·27	(1·49)	*1·04	5·01
0·4	(0·55)	0·04	1·95	(0·87)	0·30	4·66	(1·38)	3·16	11·31
12·5	(0·20)	0·70	2·08	(0·22)	1·66	4·41	(0·25)	3·96	9·40
44·2	(0·24)	1·35	2·69	(0·30)	1·99	5·37	(0·36)	2·95	10·84
60·0	(1·91)	0·32	2·09	(0·64)	1·48	4·47	(0·28)	6·82	9·64
For fish acclimated to 40·0‰									
40·0	(0·43)	1·29	1·51	(0·33)	1·66	4·45	(0·26)	2·09	13·03
0·4	(0·39)	0·64	1·51	(0·39)	1·32	4·42	(0·38)	2·69	12·88
12·5	(0·45)	0·39	2·02	(0·44)	1·23	4·12	(0·43)	3·83	8·51
18·0	(0·47)	0·43	0·87	(0·35)	1·41	3·94	(0·26)	4·79	20·68
60·0	(0·28)	0·50	2·27	(0·33)	1·41	4·84	(0·35)	4·03	10·47
For fish acclimated to 12·5‰									
12·5	(0·03)	0·63	1·32	(0·08)	1·91	3·89	(0·15)	7·18	11·75
0·4	(0·86)	0·51	1·00	(0·38)	1·40	3·95	(0·16)	3·80	15·85
30·4	(0·50)	0·55	1·05	(0·31)	2·07	4·39	(0·18)	7·67	18·54
44·2	(0·34)	1·14	1·18	(0·30)	2·00	5·42	(0·26)	3·50	19·32

* at 50 mm. Hg.

by a factor and drawn over that of a 5 gm. fish, in order to display the closeness of proportionate rates of uptake between 150 and 25 mm. Hg pO_2. However, this proportionality is not strictly maintained in the instant rates in other salinities with the exception of the instant rates in 0·0 and 12·5‰ of which the former is a lethal medium and the latter is not. The two sets of curves indicate some degree of proportionality with the difference that in the former, there is greater dependency on the pO_2 than in the latter case.

In 12·5 and 44·2‰ all the sizes display a relatively greater ability to utilize oxygen in the lower pO_2. This ability is probably related to the incipient lethal level because at 25 mm. Hg pO_2 the fish is close to the lethal point. Assuming this to be the case, then, it can be stated that a higher rate of uptake or greater ability to utilize oxygen in the lower concentrations of oxygen in the medium is, within the viable range of salinity, related to a reduction in the standard metabolism; because the latter and the incipient lethal level are closely linked (Fry, 1947). In regard to the instant rates in 60‰ the irregularity of the curves are exaggerated and the size differences are more obvious. The flatness of the curve of the 45 gm. fish in 60‰ may again indicate that on this size the effect of the concentrated medium is not much and the fish is able to use oxygen with greater ease even in the lower pO_2. As was demonstrated with reference to the milk fish (Job, 1957) the curves reviewed here also reflect the intensity of metabolism of the different sizes and it appears that within the viable range of salinity and where no

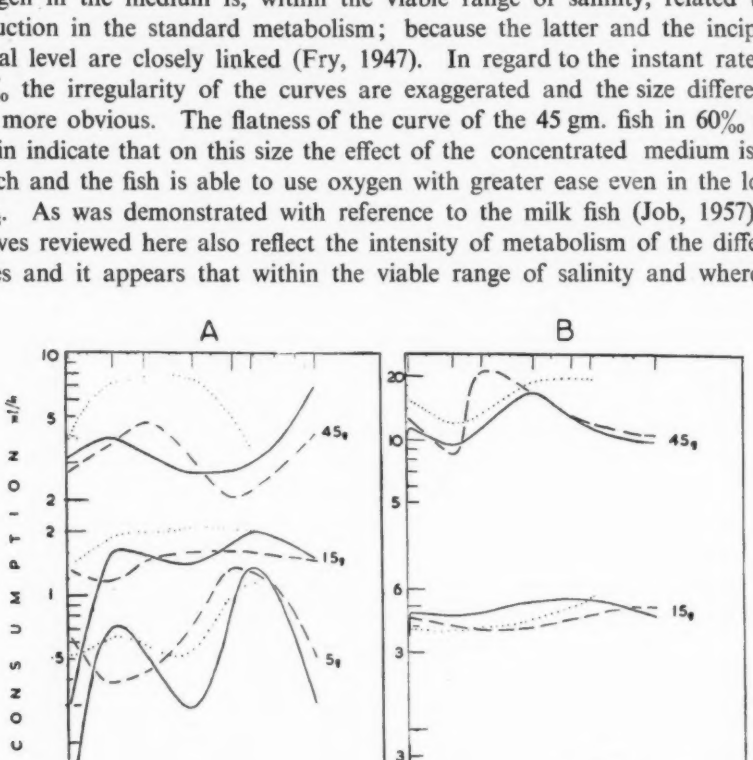

FIG. 4. Active metabolism of a 5, 15 and 45 gm. fish acclimated to salinities of: 30·4‰ (continuous line); 40·0‰ (broken line) and 12·5‰ (dotted line). Panel A displays the rate of oxygen consumption when the pO_2 is 25 mm. Hg and Panel B when it is 150 mm. Hg. The respective weights for the three sets of curves are indicated against each set on the right.

accessory factor interferes, the proportionate ability to utilise oxygen remains the same for all sizes in the range tested, under all pO_2 in the medium.

High salinity acclimation (40‰): Figure 5 B contains the curves for the dependent rates of respiration when fish were acclimated to a salinity higher than that in S.W. In this acclimation both the normal rates and the instant rates show the greatest variations. The normal rates show that there is no proportionality of response under all partial pressures of oxygen. The critical point (where the curve drops sharply) for the 5 gm. fish lies near 50 mm. Hg pO_2 whereas it is even at 150 mm. Hg for the 45 gm. fish. This indicates the intensity of metabolism in the two sizes. The bigger fish has to expend energy and consume oxygen at a faster pace in order to maintain osmotic balance with the environment, whereas the 5 gm. fish has been able to do the same even as the pO_2 reached 50 mm. Hg. The curves of the instant rates in the other salinities further reveal the inherent size differences. However, it must be mentioned that the instant rates in 18‰ showed that the 45 gm. fish consumed oxygen when the pO_2 was 150 mm. Hg, at a rate which was the highest recorded for the entire series exceeding the normal rates in 12·5 and 40‰ by a wide margin and the normal rates in S.W. by a narrower margin. On the contrary the 5 gm. fish recorded the opposite trend in that salinity. As in the previous case of S.W. acclimated fish, the extremes of salinity appear to obliterate size differences in the proportionate ability to utilize oxygen under all pO_2 so that the curves appear similar to one another.

Low salinity acclimation (12·5‰): The most striking feature of the curves in this series is the close resemblance of the respective curves of the normal and instant rates. The closeness of the proportionate ability to utilize oxygen under all pO_2 for all sizes is once again illustrated in the normal rates displayed in Fig. 5 C, by the curve of the 45 gm. fish (broken line) overlapping the curve for the 5 gm. fish. As can be seen from the figure in this acclimation all sizes share to the same degree the ability to consume oxygen at a greater rate when the pO_2 is 25 mm. Hg than in any other acclimation, indicating that when fish are acclimated to this salinity they are able to utilize oxygen with greater ease even in low concentrations of the gas in the medium. Further, within the series the 5 gm. fish displays proportionately a greater advantage over the 45 gm. fish in this ability.

As in the previous case the relatively higher rate of uptake in the lower pO_2 of oxygen can only be accounted for in the corresponding change in the incipient lethal level which is in turn definitely related to the standard metabolism. Viewed from this point then, of the three acclimation levels 12·5‰

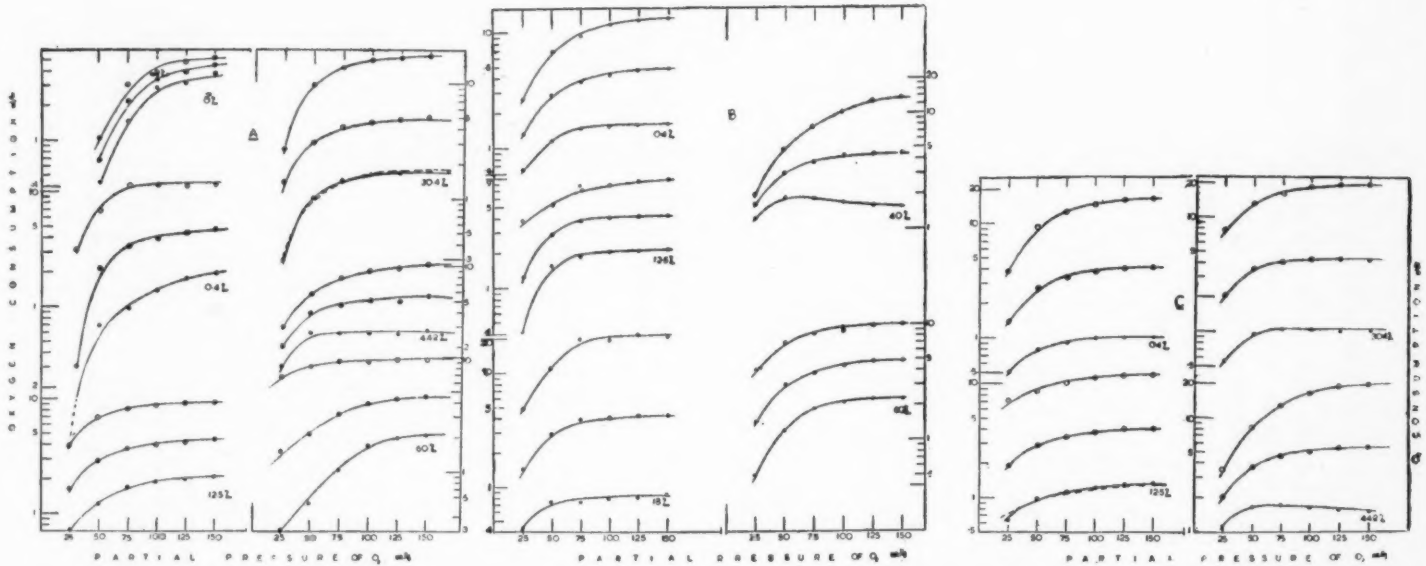

FIG. 5. Active metabolism of a 5, 15 and 45 gm. fish under various pO_2 ranging from 150–25 mm. Hg at 29° C. The open circles are the rates of a 45 gm. fish, the half-closed circles those of a 15 gm. and the closed circles those of a 5 gm. fish. The salinity of the experimental medium is indicated on the right below each set of curves. A—for fish acclimated to 30·4‰, B—to 40‰ and C—to 12·5‰ salinity.

is a medium which confers on the fish of all sizes and particularly the 5 gm. fish greater ability to accommodate salinity change. It is somewhat like a neutral medium in which the regulating mechanism itself is not geared to any particular kind of osmotic work but stays in a plastic condition capable of adjustment to higher or lower salinity within the viable range, with equal facility and ease. Partial confirmation of the above is to be found in the following section where the correlation between the different acclimation salinities and the lethal level of the different sizes of fish is traced.

Salinity and Lethal Oxygen Levels: The values obtained for this series of measurements are given in parenthesis in Table I, and are displayed in Fig. 6. The lethal levels of fish acclimated to 30·4‰ (S.W.) and tested in

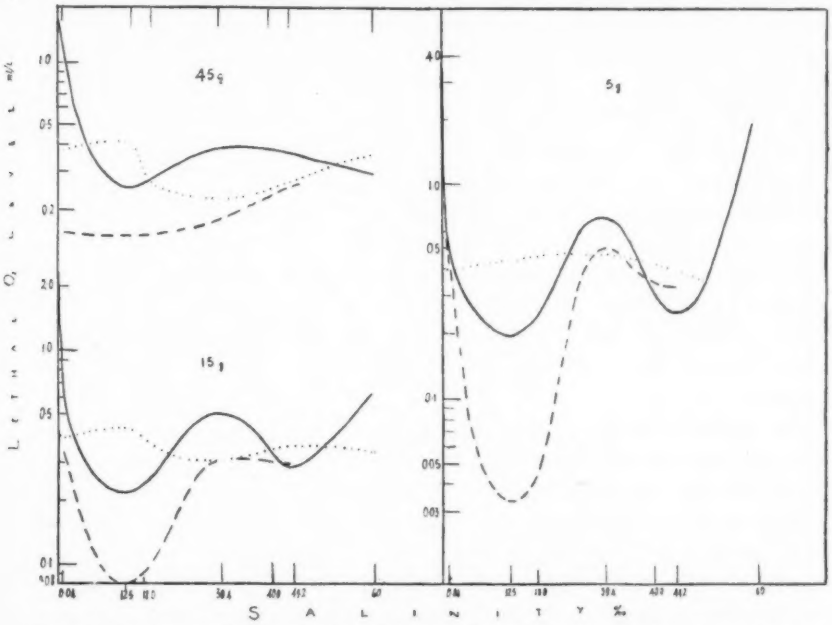

FIG. 6. Data illustrating the salinity-lethal level of oxygen relationship in a 5, 15 and 45 gm. fish acclimated to 29° C. and to salinities of 30·4‰ (continuous line), 40·0‰ (dotted line) and 12·5‰ (broken line) and tested at that temperature and in different salinities. The weights are indicated above the respective sets of curves.

the various salinities is shown by the unbroken line in the figure. One common feature shared by fish of all sizes is that the lethal level in 12·5‰ is depressed below even that in the natural environment of 30·4‰. It has been pointed out already that the ability to utilize oxygen even in the lower partial pressures of the gas in the medium was relatively high for all sizes in 12·5‰

and now the close relationship between that ability and the depression in the lethal level is clear.

Further close examination would indicate that there are inherent differences in the curves according to size in that the 5 and 15 gm. fish displayed a second depression in the lethal level in the region of 40‰ but not the 45 gm. fish. A comparison between the lethal levels in S.W. and those in 12·5 and 44·2‰ in the series shows that in the 5 gm. fish it falls from 0·7–0·2 and 0·24 ml./lit. and in the 45 gm. fish from 0·38–0·25 and 0·36 ml./lit. respectively; the difference being proportionately reduced as the size increases.

The curves indicated by the dotted line (Fig. 6) correspond to the lethal levels obtained for the three sizes when acclimated to a salinity of 40‰. In this series the highest lethal level for the 5 gm. fish was 0·47 ml./lit. in 18‰ and the lowest 0·28 ml./lit. in 60‰. The 15 gm. fish showed the highest value of 0·44 ml./lit. in 12·5‰ and the lowest in 40 and 60‰ amounting to 0·33 ml./lit. For the 45 gm. fish on the other hand the levels were 0·43 ml./lit. in 12·5‰ and 0·25 ml./lit. in 40‰. The most remarkable feature is that the effect of acclimation to the higher salinity (40‰) does not confer any special advantage to any of the three different sizes especially when these levels are compared with the corresponding levels in the salinity of their natural environment: S.W. 30·4‰. At the higher acclimation, the lethal level of the 5 gm. fish was reduced by 38·6%, that of the 15 gm. fish by 35·3% and that of the 45 gm. fish by 34·2% over the corresponding levels in S.W., for fish acclimated to that salinity. Thus the effect of acclimation to higher salinity appears to be uniform for all sizes as far as the lethal level is concerned.

In Fig. 6 the broken lines represent the curves for the lethal level of the three sizes when acclimated to a salinity of 12·5‰. Under this acclimation the 5 and 15 gm. fish displayed the same general trend that was noticed in the S.W. acclimated fish but the depression or elevation in the lethal level varied in intensity. Confirming what was already noted in S.W. fish the lethal level in this acclimation was lower for all sizes than in any of the other two cases of acclimation; and the depression in 12·5‰ itself was the lowest recorded in the entire series. When compared with the S.W. acclimated fish the lethal levels in 12·5‰ fell by 85%, 64% and 4% in the 5, 15 and 45 gm. fish respectively over the corresponding values in S.W. Thus the greatest effect according to size was noticed in the smallest fish. Another aspect of the figure is that the curves for the different sizes in this acclimation have a tendency to be lower in value in relation to the corresponding levels of S.W. fish in the zone of salinities below 30·4‰ and to be higher in the salinities above that, within the ranges tested.

From the above information and from the table given by Black (in Hoar *et al.*, 1951), it is possible to piece together an interesting correlation. In that table (p. 53) she has given the freezing point depression values for the blood of teleosts with the corresponding equivalents in salinity values. By re-plotting the data graphically a straight line relationship was obtained and it was possible from it to estimate that the blood of teleost will be in osmotic equilibrium in salinities between 7·5 and 14‰ approximately. It is to be expected then, that in these salinities naturally, the cost of regulation will be least in terms of the energy expended. Further, it will be more advantageous for a fish whose powers of regulation are limited or are still in the formative stages and is relatively 'poikilosmotic' to remain in a medium where it is in osmotic balance with the environment than for a fish whose powers of regulation are well organised and which is relatively 'homoiosmotic' since the latter will have to expend energy in any case to keep its regulating mechanism running. As the former will behave for all practical purposes like an osmometer the metabolic stress due to osmoregulation will be least when there is osmotic balance between the inner and the outer media (*cf.* Schlieper, 1929). This concept could be extended in interpreting the differences seen according to size in the lethal level of a 5 gm. fish whose survival value in 12·5‰ is greatly enhanced probably because that salinity is in osmotic balance with its internal *milieu*. The advantage for the 5 gm. fish is quite out of proportion to that of the 45 gm. fish which is capable of regulation and is actively regulating and expending energy and no marked change in its lethal level is seen. The advantage gained is in the order of 85% for the 5 gm. fish and only 4% for the 45 gm. fish.

It is thus possible that by adjusting the salinity of the water in which fish fry are transported to such levels when the osmotic stress on the fish will be least, their survival value particularly with reference to oxygen requirements could be enhanced. For *Plotosus* and perhaps for most marine species salinity of the order of 12·5‰ would appear to meet that condition. Fish fry dealers in Japan and in China have been using a slightly saline water for transporting fish fry apparently without knowing the precise physiological implications (Sproston, personal communication). Various chemicals (Srinivasan *et al.*, 1955) and drugs (McFarland, 1954) have also been used in this type of investigation and the results indicate that the chemicals intended to alleviate one condition impose upon the fish their own effects and create a complex situation, defeating the very purpose for which they were meant.

The advantage of the above method of reducing the osmotic stress on the animal and through it the respiratory metabolism has the distinct advantage of having no ill-effects on the animal and of being easily reversible

so that they can become normal when returned to the original medium. However, a precise knowledge in this regard of the requirements of the individual species employed on a large scale in fish culture would greatly add to the success of transportation or planting of the fry of the species.

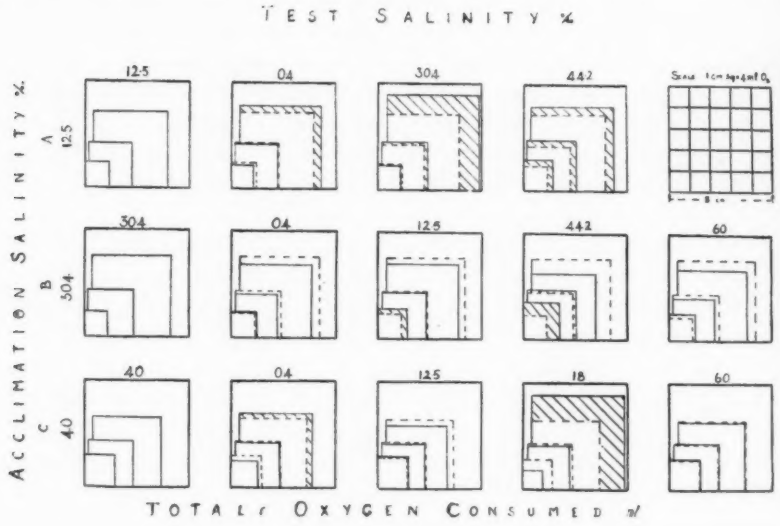

FIG. 7. Histogram illustrating the absolute and reciprocal effects of different salinities for fish weighing 5, 15 and 45 gm. For details please see text.

The relation of salinity and oxygen consumption dealt with above will remain incomplete without reference being made to the standard metabolism in the different salinity acclimations. The following section presents that aspect of the study.

Standard Metabolism and Salinity: The standard metabolic rate was measured at 29° C. with fish of average weight of 7·26 gm. acclimated to that temperature and to salinities of 12·5‰ and 30·4‰ and tested in the respective media. The S.W. (30·4‰) fish gave an average standard metabolic rate of 1·104 ml./hr. and the low salinity (12·5‰) fish an average rate of 0·177 ml./hr. The percentage of fall in the standard rate in the dilute medium works out to be nearly 84%. Though not strictly comparable in weight the fall in the lethal level of a 5 gm. fish acclimated to and tested in 12·5‰ when compared with that in S.W. (30·4‰) amounts to nearly the same percentage. Similar reductions in the metabolic rate have also been recorded in other animals by Lofts (1956) and by Potts (1954).

These figures prove convincingly that the cost of osmotic (ionic) regulation is quite significantly manifested in the metabolism of the fish. The statement made earlier that the lethal level of oxygen and the standard metabolism are directly proportional to each other is equally well established especially with reference to the particular size and the salinity in question.

Hayes (1930) suggested that the different responses in oxygen uptake in relation to salinity of the environment are positively related to changes in the standard metabolism. Such a change according to Fry (personal communication) can be brought about by a true reduction in the cost of osmotic regulation or a change in the blood ionic content sufficient to alter the standard metabolism. Even though it might be rather difficult to separate the one from the other, at least in regard to the smaller fish it has been definitely indicated that there is a substantial reduction in the standard metabolism especially in 12·5‰ salinity when isotonicity is probably reached between the fish and its environment. Veselov (quoted by Black, 1951) recorded a 60% reduction in the oxygen uptake in 10–15‰ salinity in the gold fish which indicates the proximity of the range between a marine and freshwater fish.[1]

It is evident from the above that there is a positive relationship between osmotic regulation and metabolism. But at the same time it is equally uncertain how if osmotic work turned out by the fish could be a function of the surface area of the gills or of the body, the curves relating metabolism to salinity (Figs. 1–3) could exceed the $\frac{2}{3}$ proportionality of surface rule or the slopes relating growth of the gill to the growth of the entire animal (*see* review by Fry in Brown, 1957).

Absolute and Reciprocal Effects of Salinity: In the various sections dealt with earlier much of the subject related to a qualitative estimate of the response in the metabolism to the different salinities. The data lend themselves to a quantitative estimate also being made of that relationship. The rates of oxygen consumption are converted into total oxygen consumed during the experiment and the values obtained are presented in Table II and in Fig. 7. In the figure the largest of the squares represent the total oxygen available to the fish and is equal to 100 ml. The three remaining squares in each set represent the actual total oxygen consumed by the fish: the smallest representing the 5 gm., the intermediate square the 15 gm. and the next bigger the 45 gm. fish. The scale is indicated in the square at the right-hand top corner of the figure. The series A, B and C represent the normal

[1] Low salt concentration therefore does not always "stimulate" the basal metabolism as suggested by Wikgren (1953) (p. 74).

TABLE II

Normal and instant metabolism of a 5, 15 and 45 gm. fish

Exp. sal ‰	Total oxygen consumed (ml.) by a fish		
	5 gm.	15 gm.	45 gm.
For fish acclimated to sea-water (30·4‰)			
30·4	5·50	19·15	57·18
00·4	5·75	16·18	47·55
12·5	7·93	18·15	49·00
44·2	12·30	22·00	38·18
60·0	6·65	16·50	44·50
For fish acclimated to 40‰			
40·0	8·25	18·50	42·50
00·4	6·50	18·12	48·43
12·5	8·62	17·25	34·93
18·0	3·50	16·68	79·25
60·0	8·81	18·93	40·87
For fish acclimated to 12·5‰			
12·5	5·52	16·88	50·68
00·4	4·56	16·50	60·18
30·4	4·70	19·56	78·62
44·2	8·35	21·75	62·32

metabolism of fish acclimated to 12·5, 30·4 and 40‰ salinity respectively and the others read horizontally against the three, the corresponding instant metabolism in the respective test salinities indicated therein. The broken lines in the instant metabolism squares indicate the respective normal rates drawn for the sake of comparison. Wherever this area is shaded it indicates the extent to which the instant exceeds the normal and where it is left unshaded the opposite condition is indicated.

In brief the data indicate that (1) the normal metabolism of the 5 gm. fish is more or less the same in both 12·5 or 30·4‰ but it is not true of the other two sizes; (2) that the change in the instant metabolism when compared with that in the normal indicates opposite trends between the 5 and 45 gm. fish and the rise or fall in either case being remarkably close, *i.e.*, + 44·18 and − 14·8% in the 5 gm. and − 14·25 and + 55·1% in the 45 gm. fish; (3) the intrinsic changes of the 5 gm. on transfer from 12·5–30·4‰ is lower than that in the opposite direction and *vice versa* in the 45 gm. fish; (4) similar comparison between the values obtained between salinities of 12·5 and 40‰ indicates true reciprocity in the case of the 45 gm. fish (− 18 and + 22·9%) while the 5 gm. fish registers an increase either way, the change from the lower to the higher salinity being ten times higher than that in the opposite direction.

In this context the observations on the killifish (Keys, 1931 and 1933) and on young migrating (elver) and adult eel (Raffy, 1933) are of interest. Within the same species the effect of salinity varies in relation to size and in this particular species within the size range studied there is no true reciprocity in all cases. The greater powers of increasing the metabolic rate by the smaller fish is probably a compensating device for the absence of a more efficient osmoregulatory system. Likewise the greater ease of adaptation to a lower salinity (12·5‰) as against the higher (40‰) is also related to the fact that isotonicity being probably reached in the former case, the smaller fish expends proportionately less oxygen for osmoregulation. This capacity has already been demonstrated in the standard metabolism of the smaller fish and has been indicated in the flounder by Chaisson (1931) and in other animals by Raffy (1932) and Topping and Fuller (1942). It is also seen that the ability to utilize oxygen when the latter is near the lethal zone (25 mm. Hg) is of a higher order in the 5 gm. fish than in the 45 gm. fish amounting to 51·1 and 34·7% of the rates at 150 mm. Hg pO_2 respectively as calculated from the data presented in Fig. 5.

This is an important factor in the distribution of the species in that while the adults have never been observed in the creeks and lagoons of this area when the salinity is low, the young ones did occur there.

Summary

The metabolism of *Plotosus anguillaris* (Bloch) a marine cat-fish weighing from 5–45 gm. was studied at 29° C. after they were acclimated to that temperature and to salinities of 12·5, 30·4 (S.W.) and 40‰. The salinity of the experimental medium ranged from 0·4–60‰ in the active metabolism

experiments and were 12·5 and 30·4‰ in the standard metabolis mexperiments.

When the active metabolism of the three sizes is related to different salinities (at pO_2 of 150 mm. Hg) the 5 and 45 gm. fish showed opposite trends. In spite of the intrinsic differences both the 'normal' and 'instant' metabolism follow the same general pattern for fish acclimated to 12·5 and 30·4‰ and that the proportional ability to utilize oxygen also remains the same for all sizes at all pO_2 as far as the normal rates are concerned in those two salinities. However this is not so in 40‰ salinity.

The lethal oxygen level showed distinct size-salinity differences in that the lowest level was obtained in 12·5‰. A 5 gm. fish showed an 85% reduction in its lethal level over that in S.W. and no comparable reduction was noted in that of the 45 gm. fish. There was no change in the lethal level of fish of either size when acclimated to 40‰ and similarly compared.

It is demonstrated through appropriate experiments that the ability of the fish to utilize oxygen at greater rates even in low (25 mm. Hg) pO_2 within the viable range of salinity is related to a lowered standard metabolic rate and through it to a lowered lethal level especially in 12·5‰ salinity.

The absolute and reciprocal effects of salinity on total oxygen consumed shows that the smaller fish displays greater tolerance to low salinity and greater flexibility in changing its metabolic intensity as with changing environmental salinity. The absence of this ability in the 45 gm. fish is probably made up by a more efficient osmoregulatory system.

The distribution of the species according to size and salinity and the problem of live-fish transport are discussed.

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BREEDING RICE VARIETIES RESISTANT TO BLAST DISEASE CAUSED BY *PIRICULARIA ORYZÆ* CAV.

I. Selection of Resistant Varieties from Genetic Stock

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Introduction

A programme of selecting rice varieties resistant to blast disease (*Piricularia oryzæ* Cav.) was initiated at the Central Rice Research Institute in 1948. In carrying out the tests for resistance, methods were standardised for bringing about artificial infection, scoring infection and classifying the varieties into different susceptibility groups. The procedures adopted in these tests and the results obtained in the first phase of the programme completed in 1955, are presented below.

Materials and Methods

Out of the collection of genetic stock maintained at this Institute, which consisted of over two thousand types in 1948, four hundred and seventy types were taken up for the tests. These were mostly improved varieties released for cultivation by various rice research stations in India and also a few foreign varietes.

The method of testing consisted of a series of screening tests in the seedling stage under artificial infection, followed by rigorous tests in the field under conditions favourable for blast development. In addition, all these types were also observed for their reaction to the natural infection of blast at seedling, post-transplanting and flowering stages in maintenance plots.

Artificial infection in the seedling stage.—A unit of twenty-five seedlings was raised for each variety in five small pots of 6 inches diameter, using normal field soil mixed with a small amount of well rotten farm-yard manure. When the seedlings were 25-30 days old, ammonium sulphate to give approximately 40 lb. of nitrogen per acre was applied to the pots. Within a week the seedlings begin growing vigorously with leaves turning dark-green when they are considered ready for artificial infection. In the first artificial infection test carried out in 1949, an isolate of the fungus obtained from Coimbatore was used. In the subsequent years, an isolate from the Institute

farm was used. No evidence for the existence of specialisation in pathogenecity was obtained in an infection experiment carried out in 1949 with isolates of the pathogen from different centres, Coimbatore, Wynad, Cuttack and Delhi (Padmanabhan, 1953 *a*).

The spore suspension was prepared from a culture of the fungus in oat meal agar containing thiamine (0·25 mg. in 1000 ml.) and biotin (0·015 mg. in 1000 ml.) and incubated at about 25° C. for 7–12 days. The suspension was prepared with sterile water or a solution of gelatine 0·25% *plus* sodium oleate 0·05% (Anderson and Henry, 1946). The seedling leaves were first wetted with water from a sprayer, and then sprayed with spore supension to run-off by using Devilibis atomiser.

For carrying out artificial infection, the pots were first arranged in compact blocks of 400 each, each block being covered all round with a wet cloth curtain suspended from a wooden frame about 6 feet high to provide the humid chamber conditions. The infection was carried out after sunset, and the seedlings were kept enclosed in the wet cloth chamber till about 8 A.M. the next morning. These precautions were taken to ensure contact of the leaves with water for as long a period as possible, as contact with water for a minimum period of 6–10 hours has been found to be necessary for establishment of infection by *Piricularia oryzæ* (Hemmi and Abe, 1931; Anderson *et al.*, 1947). During the period of incubation, the temperature ranged from 25–27° C. within the enclosure of the cloth curtain.

Index for the type of spots		Index for the number of spots
A. Just reddish flecks		
B. Minute reddish spots showing no differentiation into distinct zones	I	1–3 spots per leaf
	II	4–15 spots per leaf
	III	Above 15 spots per leaf
C. Circular spots about 2–3 mm. in diameter with a central ashy zone and a dark purplish brown margin		
D. Broadly spindle-shaped spots only slightly longer than broad, 3–5 mm. in diameter		
E. Large distinct spindle-shaped spots with a central ashy zone and marginal zones 3–5 mm. broad and up to several centimetres in length		

The first sign of infection could be seen on the seedling leaves after 48 hours in some varieties, and within about a week the spots attained their maximum development. The leaf spots varied in size from small minute flecks to large spindle-shaped spots. The number of spots also showed some variation. Therefore, in scoring for infection, the extent of development of the individual spots as well as the number of spots which developed on a leaf were taken into consideration. Accordingly, a score card was devised as follows (Fig. 1).

With the help of the above score card the infection observed on the most heavily infected leaf in each seedling was scored as "II D" or "I C", etc. Then the observed infection score was converted to a numerical scale as follows:—

Numerical value for the type of spot		Numerical value for the number of spot	
A	1	I	2
B	2	II	5
C	4	III	10
D	8		
E	16		

The product of the two values for the type of spots and their number was taken as the numerical score. For example, if the infection score of a leaf was II C or III D, the corresponding numerical score was $5 \times 4 = 20$ or $10 \times 8 = 80$.

Further, in the case of very heavy infection seen on some susceptible types, an additional weightage was given for the leaf area totally destroyed, and a numerical value allotted to the percentage of leaf area destroyed, as below, was added to the numerical score of the leaf.

30% of leaf area destroyed	10
50% of leaf area destroyed	20
75% of leaf area destroyed	30
The whole leaf totally destroyed	40

The average infection score of the variety was arrived at by totalling the individual infection scores of the seedlings and dividing the total by the number of infected seedlings.

In the seedling infection tests, the varieties which received an infection score of 10 and above or which had any " D " or " E " type of spots were classed as belonging to the susceptible groups in the seedling stage.

Field tests.—The field tests were carried out for three successive years with the varieties carried forward from the screening tests, *i.e.*, varieties which had emerged as resistant both from the seedling tests and the type maintenance plots in the field. In each successive field tests, only the varieties which were resistant in the previous tests were included.

The seedbeds were sown in the first week of July. When the seedlings were three weeks old, ammonium sulphate was applied to the beds at the rate of 40 lb. N per acre. Observations on disease incidence in the beds were taken at the time of uprooting of the seedlings in the first week of August.

The seedlings were transplanted in a well prepared puddle. Green manure and farm-yard manure were applied as a basal dressing to give about 20 lb. N per acre. Three to four weeks after transplanting, ammonium sulphate was applied at the rate of 40 lb. N per acre.

In the first field test carried out in the year 1953–54, the test varieties were transplanted in two rows of 20 feet each, with a spacing of 6 inches between the rows and 6 inches between the plants in the row. In subsequent tests, each variety was transplanted in five rows of 20 feet each, with one foot of space between the rows and 6 inches between the plants in the row. The wide spacing between the rows was found useful for taking observations. A row of the highly susceptible variety Co. 13 was planted on either side of the test variety. Each variety was replicated twice. The total number of plants tested for each variety was 160 in the first test and 400 in subsequent tests.

Observations on the disease development in the field were taken twice in the season the first on the foliar infection at the rapid tillering phase and the second on neck and nodal infection at the time of the harvest of the variety. For leaf infection, the most heavily infected leaf in each plant was scored. At the time of harvest, the number of plants which had developed neck infection and nodal infection, and the number of healthy and infected tillers in each of the affected plants were noted. From the data the percentage of plants which had developed neck and nodal infection was calculated.

As blast appears under Cuttack conditions both in the seedbeds and in the transplanted crop in the tillering phase during the second crop season, also (January–April) the reaction of the test varieties to the disease in the second crop season also was studied more or less on the same lines as in the first season.

The criteria adopted for classification of the varieties were as follows:

Very resistant ..	Leaf-infection score less than 10, 'C', 'D', 'E' spots absent, neck-infection less than one per cent.
Resistant ..	Leaf-infection score less than 10, 'C' spots seen very rarely, 'D' and 'E' spots absent, neck-infection one to five per cent.
Moderately resistant ..	Leaf-infection score less than 10, 'C' spots common, 'D' and 'E' spots absent, neck-infection six to ten per cent.
Moderately susceptible	Leaf-infection score less than 20, 'E' spots absent neck-infection eleven to twenty per cent.
Susceptible ..	Leaf-infection score above 20, 'D' and 'E' spots common, neck-infection more than twenty per cent.

Results

On the basis of their reaction in the artificial infection tests in 1949, 1950 and 1951 and their field reaction in the type maintenance plots during 1948–53, three hundred and eighty-seven test varieties were eliminated as susceptible. In the field tests carried out during 1953–54, 1954–55 and 1955–56, five varieties were finally selected as resistant and sixteen as moderately resistant. The names of the selected varieties are given below.

Resistant ..	BJ. 1, CO. 4, S. 67, SM. 6 and SM. 9
Moderately resistant ..	ADT. 12, AKP. 8, AKP. 9, AS. 2, BAM. 4, CH. 55, CO. 25, CO. 26, CP. 6, CP. 9, H. 755, MTU. 5, Mugad-249, PTB. 10, S. 624, and SM. 8.

Out of the selected varieties, CO. 4, CO. 25 and CO. 26 have already been selected as resistant to blast by the Department of Agriculture, Madras, and Mugad-249 selected by the Department of Agriculture, Bombay. The reaction of the two varieties, PTB. 10 and S. 624 was found to be somewhat variable in some seasons being moderately susceptible or susceptible to leaf-infection or neck-infection.

The list of varieties tested and their classification into different relative susceptibility groups are presented in Table I.

TABLE I

Reaction of rice varieties to blast disease (tested at Central Rice Research Institute, Cuttack, during the years 1948–55)

Place of origin	Very resistant	Resistant	Moderately resistant	Moderately susceptible	Susceptible
1	2	3	4	5	6
India:					
1. Andhra ..			AKP. 8 (Maharajabhogam), AKP. 9 (Bangarutheega), MTU. 5 (Krishnakatukulu)	AKP. 6 (Mypali), BS. 1, HS. 21, MTU. 6 (Atragada)	AKP. 2 (Sunkisannam), AKP. 5 (Mypali), AKP. 7 (Palgarabayyahunda), AKP. 10 (Bangarutheega), AKP. 11 (Ramasagaram), HS. 8, HS. 12, HS. 19, HS. 22, HS. 35, HS. 38, HS. 39, HS. 47, MTU. 1 (Akkulu), MTU. 2 (Akkulu), MTU. 3 (Basangi), MTU. 4 Basangi), MTU. 7 (Guttikusuma), MTU. 8 (Vankisannam), MTU. 9 (Garikasannavari), MTU. 10 (Krishnakatukulu), MTU. 11 (Konamani), MTU. 12 (Pedhaatragada), MTU. 13 (Delhibhogam), MTU. 14 (Atragada), MTU. 15, MTU. 16, MTU. 18, (Kodijillama), MTU. 19, SLO. 1 (Punasakonamani), SLO. 2 (Punasakonamani), SLO. 3

(Konamani), SLO. 4 (Kona-

			(Konamani), SLO. 4 (Konamani), SLO. 5 (Palagummsari), SLO. 6 (Punasa-akkulu), SLO. 8 (Sannabasangi), SLO. 9 (Gortibasangi), SLO. 10 (Ratnachudi), SLO. 11 (Bikirisannam), SLO. 12, SLO. 14 (Punasaakkulu), SLO. 17, SLO. 18
2. Assam ..	AS. 2 (Kasalath)	S.55 (Badshabhog), S. 126 (Laundumra), S. 156 (Nagrasail), SB. 279 (Gamiribora)	AR. 1 (Salibadal), AR. 108-1 (Dholabadal), AR. 353-148, AS. 20-1 (Garen), AS. 35 (Farmah), HBJ. 1, HBJ. 2, HBJ. 3, HBJ. 4, M. 36-30 (Baurashmurali), M. 142 (Koimurali), M. 175-1 (Dholajali), S. 22 (Latisali), SC. 54-60 (Vijoysali), SC. 94-47 (Kerrsali), SC. 412-56 (Swarnasali), SJ. 226 (Bengalijoha), SL. 70 (Ahomsali), T. 2089 (Prasadbhog), T. 2090 (Andrewsali), T. 2091 Badshabhog, T. 2092 (Laundumra), T. 2094 (Jahari), T. 2095 (Latamaguri), T. 2096 (Gomiribora), T. 2097 (Karanganj), T. 2100 (Latisali), T. 2102 (Kerrsali), T. 2104 (Kasalath), D. 204-1 (Dumai), C. 203-3 (Chengri)
3. Bihar ..		BHR. 16 (Motisal), BHR. 141 (Juhlansar)	BHR. 36 (Kessore), BHR. 88 (Dahia), BHR. 115 (Dahia), BHR. 76-Bk

TABLE I—(*Contd.*)

Place of origin 1	Very resistant 2	Resistant 3	Moderately resistant 4	Moderately susceptible 5	Susceptible 6
4. Bombay ..			Mugad-249	Antersal-67, Antersal-200, Bhadas-79, Chimansal-94, Dodgya-622, Krishnasal-10, Patnai-6	Ambemohar-59, Ambemohar-157, Ambemohar-159, Antersal-90, Fine Waksal, Halga Red-244, Halga White-1690, Jaddu-1061, Kamod-86, Kolamba-184, Kolpi Early-70, Luchai, Maskaty-1315, Mugad-81, Mugad-141, Mugad-161, PR. 90, Warangal-57, Yalkirisal-4, Zinya-149, 280-51-36.
5. Kashmir ..					Bansal, Safed Zagir, Chatta Baber, Budgi, Mushka Budgi, Budgi Baber, Begam, Lolanzan, Ratibasmati
6. Kerala ..					Chuvannavellai, Kochuvittu, Karuvalan, Panamkomban, Samba, Valshivanandan, Veeravadantan, Kichilisamba, Arikirivashi
7. Madhya Pradesh			CP. 6 (Budhiabako), CP. 9 (Luchai)	CP. 2 (Nungi), CP. 8 (Benisar), CP. 12 (Banspati), CP. 13 (Kurbimohar), CP. 19	CP. 1, CP. 3 (Sultigurmatia), CP. 4 (Gurmatia), CP. 5 (Ludko), CP. 7 (Ajan), CP. 10 (Chattri), CP. 11 (Dubraj), CP. 14 (Badshabhog), CP. 15 (Chinoor), CP.16, CP. 17, CP. 18

			mohar), CP. 19	shabhog), CP. 15 (Chinoor), CP.16, CP. 17, CP. 18
8. Madras ..	CO. 4 (Anaikomba)	ADT. 12 (Chitrakali), CO. 25 (Hybrid), CO. 26 (Hybrid), PTB. 10 (Thekkan Cheera)	ADT. 6 (Red-Ottadan), ADT. 8 (Malagalukkulu White Sirumani), ADT. 9 (Poonkar), ADT.11 (Nellore Samba), ADT. 21 (Vadansamba), ASD. 6 (Anaikomban), PTB. 1 Aryan, PTB. 3 (Eravapandy), PTB. 5 (Veluthari-Kayama), PTB. 6 (Athikraya), PTB. 7 (Parambuvattan), PTB. 8 (Thavalakannan), PTB. 9 (Same as PTB. 8), PTB. 13 (Kayama), PTB.14 (Maseathi), PTB. 15 (Kavunginpoothala) PTB. 16, PTB. 18, (Erayapandy)	ADT. 1 (Red sirumani), ADT. 2 (White Sirumani,) ADT. 3 (Kuruvai), ADT. 4 (Kuruvai), ADT. 5 (Nellore Samba), ADT. 7 (Muthusamba), ADT. 10 (Korangusamba), ADT. 13 (Sannasamba), ADT. 14 (Vellaikar), ADT. 15 (Mutant from ADT. 4), ADT. 16 (Konakuruvai), ADT. 18 (Vellaikuruvai), ADT. 19 (Sarapalli), ADT. 20 (Hybrid ADT. 3× ADT. 2), ADT. 22 (Vadansamba), ASD. 1 (Karsamba Red), ASD. 2 (Karsamba White), ASD. 3 (Veedivadangan), ASd . 4 (Kuruvaikalyan), ASD. 5 (Karthigasamba), ASD. 7 (Karsamba Red), CO. 1 (Natural cross from GEB. 24), CO. 2 (Poombalai), CO. 3 (Vellaisamba), CO. 5 (Chinnasamba), CO. 6 (Sadaisamba), CO. 7 (Sadaisamba), CO. 9 (Karsamba Red), CO. 10 (Gobikar), CO. 11 (Ayansamba), CO. 12 (Sendhinayagam), CO. 13 (Arupathankodai), CO 14 (CO. 3 × Burma variety), CO. 15 (GEB .24×ADT. 10), CO. 16 (same as CO. 15), CO. 17 (Vadansamba), CO. 18 (Vellaikar), CO. 19 (Sirumani), CO. 20

TABLE I—(*Contd.*)

Place of origin 1	Very resistant 2	Resistant 3	Moderately resistant 4	Moderately susceptible 5	Susceptible 6
					(Tella Sannavadlu), CO. 21 (Arupathanasmba), CO. 22 (Manavari), GEB. 24 (Mutant in Konamani), PLR. 2 (Chitrakali), PLR. 7, PTB. 2 (Ponarvan, PTB. 12 (Chitteni), PTB. 17) (Jeddu Halliga), PTB. 19 (Sirumani), PTB. 20 (Chitteni)
9. Mysore ..		S. 67	S. 624 (Maharajabhogam)		B. 16 (Thogarina), B. 194 (Musali), B. 281 (Belikannamhegge), B. 805, B. 888, B. 986, H. 324, H. 419, H. 535, S. 54, S. 139 (Mysore Kaddi), S. 199 (Alur Sanna), S. 246 (Nagpur Sanna), S. 317 (Halubbalu), S. 328, S. 476, S. 590, S. 661 (Coimbatore Sanna), S. 699 (Coimbatore Sanna), S. 701 (Coimbatore Sanna), S. 749 (Rathnachudi)
10. Orissa ..			BAM. 4 (Bayyahunda)	D.I. 3 (Orozosporas), T. 90 (Machakanta), T. 608 (Basabati), T. 1118,	BAM. 1 (Boroponko), BAM. 2 (Boroponko), BAM. 3 (Bayyahunda), BAM. 5 (Rathnachudi), BAM. 6 (Ratnachudi), BAM. 7

		T. 141 (Soru-chinnamali)	(Navakotisannam), BAM. 8 (Navakotisannam), BAM. 9 (Mypali), BAM. 10 (Mypali), BAM. 13 (Yana Kondnagi), B. 76, Benibhog, Balunganardhan, D.I. 4 (Orozosporas), FR. 13 A, FR. 43 B, ML. 1, ML. 3, N. 136, SR. 26 B (Kalambank), T. 56 (Kalakakkudia), T. 165 (Kajalchampa), T. 380 (Banko), T. 412 (Badshabhog), T. 442 (Kolakartika), T. 635, T. 812 (Rangolata), T. 885, T. 1145 (Ussa), T. 1242 (Magura)
11. Punjab ..		PB. 4 (Lal Nakanda 41), PB. 5 (Palmansuffid 246), PB. 10 (CM. 7-6), PB. 14 (Jhona 349)	NP. 97, NP. 130, NP. 137, PB. 1 (EC. 1-57-25), PB. 2 (Mushkan-41), PB. 6 (Phulputtas 72), PB. 7 (Sathra 278), PB. 12 (Mahlar 346), PB. 13 (Basmati 370)
12. United Provinces	H. 755	T. 9 (Duncapet)	Ch. 10 (UP) H. 33, H. 64, H. 108, N. 10 B (Basmati Pilibit), N. 12 (Suffaida), N. 22 (Rajbhog), N. 27 (Banki Pilibit), N. 32 (Baljati), T. 1 (Ramjiwain), T. 3 (Basmati), T. 17 (Bansi), T. 21 (Chawl), T. 22 *a* (Bansi), T. 23 (Kala Sukhdas), T. 36 (Jorhan), T. 43 (Sondhi) T. 56 (Jobda), T. 88 (Chakia), T. 100 (Benslot), T. 136 (T. 1×T. 100)

TABLE I—(*Contd.*)

Place of origin 1	Very resistant 2	Resistant 3	Moderately resistant 4	Moderately susceptible 5	Susceptible 6
13. West Bengal		Bhogjira-1		Badkalamkati-65, Manikkalma, Nagra-68-6, Bhasamanik, Nagra-41-14, Badshabhog, Kaliboro-2, Bhogjira-2	Bhutmuri-36, Badkalamkati-7, Bolder, Dahijira, Kaladubraj, Ajan 246, Nonramsail, Sindurmukhi, Raghusail, Randhunipagal, Patnai 23, Kalma-222, Rupsail, Seethasail, Kumergore Kaliboro-1, Kaliboro-3
China ..				CH. 4 (C.N.A.B. No. 4), CH. 5 (Szechuan Shui Pai Tiao), CH. 6 (Hunan Thirty culms), CH. 7 (Chekiang No. 3), CH. 13 (C.N.A.B.-Kweichow No. 2), CH. 25 (Szechuan Dan hung scented Rice), CH. 30 Hokiang Dry Field Glutinous, CH. 31 (Black Kernel Glutinous), CH. 36 (Yunan Shih Ping	CH. 1 (Canton Golden Early), CH. 2 (Nanje hao), CH. 3 (Hunan victory), CH. 8 (Chekiang No. 9), CH 9 (Rushy late 21-3), CH. 10 (C.N.A.B. 11-23-613), CH. 11 (Canton Fine leaf No. 31), CH. 12 (Canton kuan yiu hoien No. 16), CH. 14 (Kweiyaxy great white Hsleu), CH. 15 (A.H. 28-70 Faith × Fortuna), CH. 16 (A.H. 29-218 Blue Rose × Fortuna), CH. 17 (Pa-sian Red Leaf), CH. 18 (C N.A.B. Kweichow No. 8847), CH. 22 (Kunming Scented Rice), CH. 23 (Yunan we sen Serenli Perfume), CH. 26

	Purple Glutinous), CH. 40 (C.N.A.B. Land Rice), CH. 43 (974), CH. 46 (1007), CH. 49 (Linchou), CH. 53 (Wuke hualo), CH. 58 (Tainung No. 38), CH. 60 (Taoyri anyinwei), CH. 61 (Chikechin), CH. 68 (Chinshih-yih), CH. 70 (Cheanong No. 18) CH. 74 (Shentsuo No. 4), CH. 80	(Sung Pan Rice), CH. 32 (Honen Long Purple Glutinous), CH. 34 (Cheng tu Great White Fine Rice), CH. 35 (Ninger Small White Glutinous), CH. 38 (Hunan Tzu-Sin Upland Rice), CH. 39 (C.N.A.B. White Rice), CH. 42 (972), CH. 44 (988), CH. 45 (996), CH. 47 (1040), CH. 48 (Ahouchiac), CH. 50 (Paiweitsung), CH. 51 (Yinwei), CH. 52 (Chinggu), CH. 56 (Hungehiaochin), CH. 57 (Chingchiaschinggu), CH. 59 (Hsinchu No. 4, CH. 62 (Paimi-jih), CH. 63 (Tuanrzu), CH. 64 (Chinpaoyin), CH. 65 (Wuke), CH. 66 (Paimihin), CH. 67 (Tuangkuanghualo), CH. 72 (Tainong No. 45), CH. 73 (Tai-peh No. 7), CH. 77 (Kaoshiung No. 10)
Japan ..	JAP. 1 (Semichi No. 2), JAP. 3 (Kameji)	JAP. 2, (Omachi), JAP. 5(Ashahi)
New Guinea ..		New Guinea I
Pakistan ..	Atlai, Pashpai, Hashikalmi, Kagni	Indrasail, Kataktara, Dhepi, Dudsar, Charnock, Tilakka-chari, Daudkhani, Jessobalam, Panbira, Hatisail, Dharial, Jhingasail, Latisail, Pusur. Blue-stick, Daudin, Dular, Chitraj,

Place of origin 1	Very resistant 2	Resistant 3	Moderately resistant 4	Moderately susceptible 5	Susceptible 6
					Marichbati, Nigersail, Silver Jubilee, Bangalo
Russia	..			R. 4 (T. 1708), R. 5 (T. 2443), R. 9 (T. 2887), R.I.A.R.I.	R. 1 (T. 556), R. 2 (T. 1322), R. 3 (T. 1331), R. 6 (T. 2493), R. 7 (T. 2653), R. 8 (T. 2776), R. 10 (T. 3073), R. 11 (3078), R. 13 (T. 3304)
Siam	..	SM. 6 (Champa 133), SM. 9 (Pin Kaeo)	SM. 8 (Phuang Ngern)	SM. 1 (Khao Tod Long), SM. 3 (Chamba-Da), SM. 10 (Khao Bhudat)	SM. 2 (Mali Thong), SM. 4 (Nang Mol), SM. 5 (Leaung On), SM. 7 (Mali Ong), SM. 11 (Bang Phra), SM. 12 (Kod Phom), SM. 13 (Nang Tani)
U.S.A.	..			USA. 5 (Texas Patna C.I. 8321)	USA. 2 (Rexoro C.I. 1779), USA. 3 (Fortuna), USA. 4 (Nira C.I. 2072)

Discussion

In these tests artificial infection was used for rapid screening of the varieties at the seedling stage, but the final selection for resistance was based upon the reaction of the varieties in the field at the seedling, post-transplanting and neck emergence stages under conditions very favourable for the development of the disease. There were occasionally some varieties which were resistant at one stage and susceptible at another, but this differential reaction needs further careful study and confirmation. For practical purposes, it is clearly preferable to select varieties which are resistant at all stages when such varieties are available.

In the field tests, maximum advantage was taken of the prevailing climatic conditions at Cuttack to make the tests as rigorous as possible. Two factors, *viz.*, date of planting and age of the host have a definite relation to the degree of development of blast in the field (Padmanabhan and Ganguly, 1953, 1954). As regards the age of the host, maximum leaf-infection was seen on 45–50 days old plants, when they were in the vigorous tillering phase following transplanting. By delaying the planting up to the middle of August during the *Kharif* season and upto the end of January in the spring season, very heavy infection of blast could be obtained.

Since the level of nitrogenous fertilization also profoundly alters the reaction of rice varieties to blast (Krishnaswamy, 1952; Padmanabhan, 1953 *b*; Ganguly *et al.*, 1954), it was considered necessary to standardise the level of nitrogenous fertilization in these tests against blast. In the present study the level was fixed at 40–60 lb. N per acre as this level has been found to be the most economic N level for optimum yield response in rice throughout India. Besides at higher levels many a potentially good yielding variety which is not likely to develop the disease under conditions in which it will be cultivated in India will get eliminated.

Elimination of useful types is also likely to occur if the artificial infection tests are carried out under lower temperature conditions than those likely to be met with in the plains of India, since temperature influences the reaction of rice to blast through its effect on nutrient absorption and utilization (Hashioka, 1950). Conversely, varieties selected as resistant at temperature ranges common in the plains of India may not behave as resistant if they are grown under cooler temperatures. In fact, the performance of some of the varieties selected as resistant to blast in the present study in more than forty rice research centres in India during the years 1955–58 has confirmed that most of these varieties are not resistant to the disease in localities situated at higher elevations where cooler temperatures prevail.

How far the varieties selected as resistant and moderately resist ant to blast at Cuttack would be useful in different parts of India is being studied by the States in their rice research centres. The results will be published separately.

ACKNOWLEDGEMENTS

The writers wish to express their gratitude to late Dr. B. B. Mundkur for many useful suggestions and to Dr. K. Ramiah, formerly Director of Central Rice Research Institute, for his keen interest in the study. Thanks are due to Shri G. H. Chandwani for his help in the field experiments.

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BLAST INFECTION GRADES
A
B
C
D
E

STUDIES ON THE NUTRITION OF RICE PLANT (*ORYZA SATIVA* L.)

Part VII. Influence of Increasing Levels of Phosphate and Potash on Growth, Yield and Nutrient Uptake by Rice Plant (*O. sativa* var. *indica*)

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Tanaka *et al.* (1958 *a*) reported on the influence of increasing levels of nitrogen on growth, yield and nutrient uptake by rice plant. Next to nitrogen, phosphate and potash are the important major nutrients, uptake of which by plant is governed by their availability in the medium in which the plant is grown. Both these nutrients play an important part in the metabolic functions of the plant body. Sircar and Sen (1941) and Aiyer (1946), working on the role of phosphorus and Aiyer (1948) and Sircar and Dutta (1957), working on the role of potassium in rice, have reported that deficiency of both these nutrients seriously impairs growth and nitrogen metabolism in the plant. At low supplies of phosphate and potash, growth is poor and stunted, yield of grain is reduced and nutrient uptake is adversely affected.

Ishizuka and Tanaka (1951 *a* and *b*), studying the influence of increasing levels of these nutrients on growth of a *japonica* variety of rice grown under culture solution, considered 20–40 p.p.m. of P_2O_5 and 20 p.p.m. of K_2O respectively adequate for normal growth and assimilation of plant nutrients by this variety. Similar studies regarding *indica* variety of rice have, however, not been undertaken. *Indica* variety of rices are known to behave differently from *japonica* variety in respect of growth and assimilation of nitrogen (Tanaka *et al.*, 1958 *b*). Experiments were therefore undertaken to get fundamental information on the effect of increasing levels of phosphate and potash respectively on growth and nutrient uptake of an *indica* variety of rice, to know its requirement of these nutrients for normal growth. Trials were conducted in solution culture technique, under conditions free from complex heterogeneous soil influence.

Experimental Material and Methods

Rice variety *Ptb* 10 of 110 days duration was grown in water culture solution at graded levels of 0, 5, 10, 20, 40, 60, 100, 150 and 200 p.p.m. of

P_2O_5 and K_2O respectively. Technique of growing of plants was the same as described earlier (Tanaka *et al.*, 1958 *a*). Potash and phosphate series were run separately with two pots for each level of nutrient. Sodium dihydrogen phosphate and potassium sulphate were used as sources of P_2O_5 and K_2O respectively. A uniform level of 30 p.p.m. nitrogen in the form of ammonium nitrate was used in all treatments. In the phosphate series, 30 p.p.m. K_2O and in potash series, 30 p.p.m. P_2O_5 was given uniformly in the respective pots. Seeds were sown in seed pans with mixture of soil and sand on June 14 and seedlings were transplanted in pots containing eleven litres of culture solution, after fourteen days of growth in the seed pans. Culture solution was changed every week and observations on height, tiller number, etc., were taken after each change of solution. Crop was harvested on September 26 and yield data of various parts of the plant body were recorded. Plant samples were analysed for nitrogen, P_2O_5, K_2O, CaO, MgO and SiO_2.

General Observations on Plant Growth

(*a*) *Phosphate deficiency*.—Deficiency symptoms appeared in no phosphate pots, about twelve days after transplanting on July 11 and became acute by July 20. Height and tillering were both adversely affected and upper leaves of plants were narrow, short, stiff and straight. In early stages of deficiency, new leaves were bluish green and lower part of the stem was coloured with slight pinkish streaks. Later, upper leaves became deep bluish green in colour and lower leaves started drying from tip downwards. The colour of dead leaves was slightly purplish red. Roots of phosphate deficient plants were comparatively smaller in number and were coloured rust brown. Primary roots were elongated, secondary roots were very few. Flowering of phosphate deficient plants was delayed by about seven days and ear heads formed were very poor with a few number of grains per panicle.

(*b*) *Phosphate toxicity*.—About ten days after planting, on July 9, phosphate toxicity symptoms appeared in the highest phosphate level of 200 p.p.m. On July 20, 100 and 150 p.p.m. phosphate levels also showed toxicity symptoms. In early stages of toxicity, leaves lost their turgidity and leaf blades remained hanging over. Later, rust brown patches appeared on leaves, particularly on portion of the blades that were hanging over. These patches continued to increase in irregular manner and upper portions of many of the older leaves became largely rust brown in colour, but the leaves were not completely dead. Leaves were green but looked rather dried up giving appearance of higher transpiration of water from leaf surface. Flowering was also delayed by about five to eight days. Ear heads formed were light in weight with fewer number of grains per panicle.

(*c*) *Potash deficiency*.—Potash deficiency symptoms in no potash pots developed rather late, on August 15, about forty-five days after planting. Plants in 5 p.p.m. potash level also showed weak deficiency symptoms. Potash deficient plants were dwarfish, but tillering of the plants was not very seriously affected. Upper leaves were deep green, soft and small and leaf blades were wavy in appearance. The middle and lower leaves developed dark brown spots with drying at tips. Later, older leaves started drying while the younger leaves continued to be deep green. Colour of dead leaves was dirty yellow. Flowering in the potash deficient plants was delayed by about four to five days and in spite of high tillering capacity of the plant, ear-bearing tillers were few.

Experimental Results

I. Phosphate series

(*a*) *Plant height and tiller number*.—Height and tiller number were recorded at weekly intervals. It was seen that mean height of plant increased with increase in phosphate level up to 10 p.p.m. From 10–60 p.p.m., there was very little difference in the plant height, but at higher levels of 100–200 p.p.m., reduction in plant height occurred. Tiller number per pot, however, increased sharply up to 5 p.p.m. phosphate only, and at higher levels, there was very little change. Data on increase in plant height and tiller number during growth stages from planting to maturity at 0, 10 and 200 p.p.m. are represented graphically in Fig. 1.

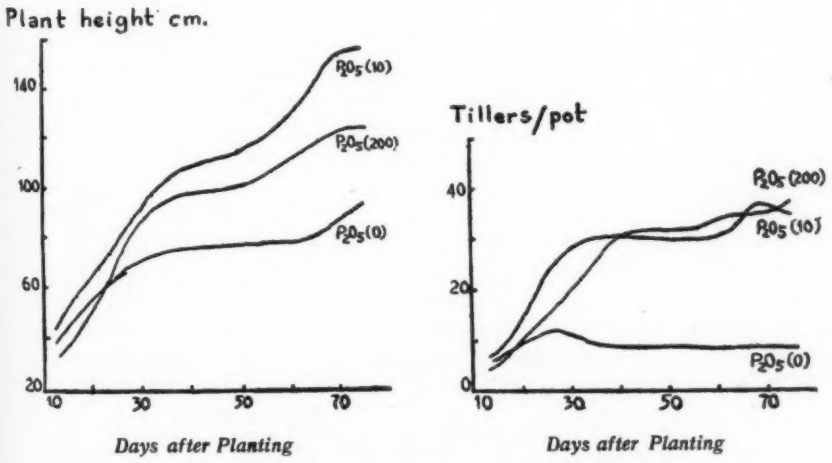

FIG. 1. Growth of rice plant at 0, 10 and 200 p.p.m. P_2O_5.

(*b*) *Plant characters and dry matter weight at harvest.*—Table I shows the influence of increasing phosphate levels on plant height, ear-bearing tillers and dry matter weight of ear, straw and root at harvest. Plant height and

TABLE I

Phosphate series—Growth character and dry matter weight at harvest

Levels of phosphate (p.p.m.)	0	5	10	20	40	60	100	150	200
Plant height (cm.) ..	92	144	155	154	152	150	142	142	123
Root length (cm.) ..	56	42	44	42	42	40	42	44	34
Ear number/pot ..	8·0	36·0	35·0	36·0	40·5	40·0	39·0	38·0	36·0
Dry matter weight (gm./pot):									
Ear	3·7	70	75	73	73	53	42	34	14
Straw	11·9	67	70	73	78	79	74	66	55
Root	4·0	12	10	10	10	9	8	7	5
Total	19·6	149	155	156	161	141	124	107	74

ear-bearing tillers are seen to follow the same trend as height and tiller number at earlier stages of growth. Root length is longest at no phosphate supply. It remains practically unaffected by phosphate levels from 5–150 p.p.m. and decreases at 200 p.p.m.

Total dry matter weight of crop is seen to increase up to 40 p.p.m. phosphate and decreases progressively at higher levels. Ear yield, however, increases only up to 10 p.p.m. and later suffers a sharp decline at higher levels beyond 40 p.p.m. Straw yield, on the other hand, increases up to 60 p.p.m. phosphate and decreases later at higher levels.

(*c*) *Mineral content of plant at harvest.*—Phosphate content of different parts of the plant is shown in Fig. 2. It is seen that phosphate content of ear, straw and root increases with increase in phosphate level, increase being particularly marked in straw at phosphate levels of 100 p.p.m. and above. Content of P_2O_5, K_2O, CaO, MgO and SiO_2 in a composite sample of the plant, containing ear, straw and root in the proportion of their respective dry weights, is shown in Table II.

Results show that increase in phosphate content of the plant is accompanied by increase in both nitrogen and potash content. CaO and MgO

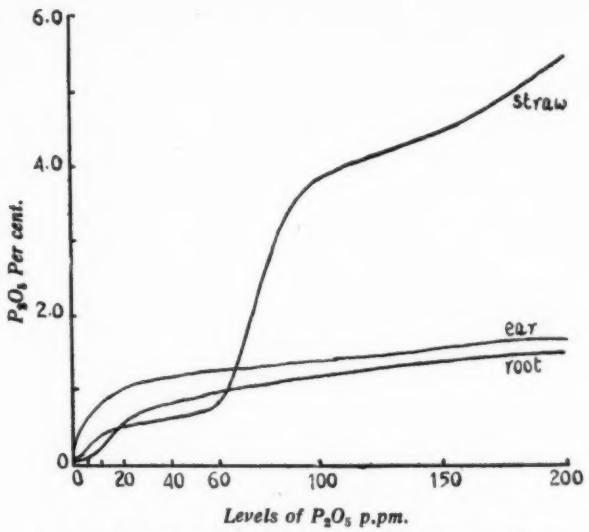

FIG. 2. Influence of phosphate level on P_2O_5 content of rice plant.

TABLE II

Phosphate series—Nutrient content of rice plant at harvest

Levels of phosphate p.p.m.	N %	P_2O_5 %	K_2O %	CaO %	MgO %	SiO_2 %
0	1·20	0·16	0·64	0·53	0·09	11·20
10	1·50	0·52	1·68	0·62	0·22	3·15
40	1·58	1·04	1·90	0·66	0·29	3·30
100	1·77	2·77	2·44	0·79	0·38	3·95
200	2·32	3·54	2·80	0·84	0·39	5·02

content also increases with increase in phosphate content, but not so markedly as the major nutrients. At no phosphate level, MgO content of the plant is very low. Silica content of the plant, on the other hand, is higher at no phosphate level, it later drops considerably at 10 p.p.m. and increases again gradually with increase in phosphate level.

II. Potash series

(*a*) *Plant height and tiller number.*—Plant height in potash series increased with potash supply upto 20 p.p.m. and was little affected at higher levels. Tiller number per pot, on the other hand, was very little affected by change in potash level. At earlier stages of growth, from three to five weeks after planting, plants grown on 0 and 5 p.p.m. potash, however, showed generally less tiller number per pot than plants at higher potash levels, but at later stages of growth, these plants caught up with the others in tiller number and at flowering, no potash plants showed even slightly more total tiller number than higher level potash plants. Figure 3 shows increase in plant height and tiller number from planting to maturity at 0 and 20 p.p.m. potash.

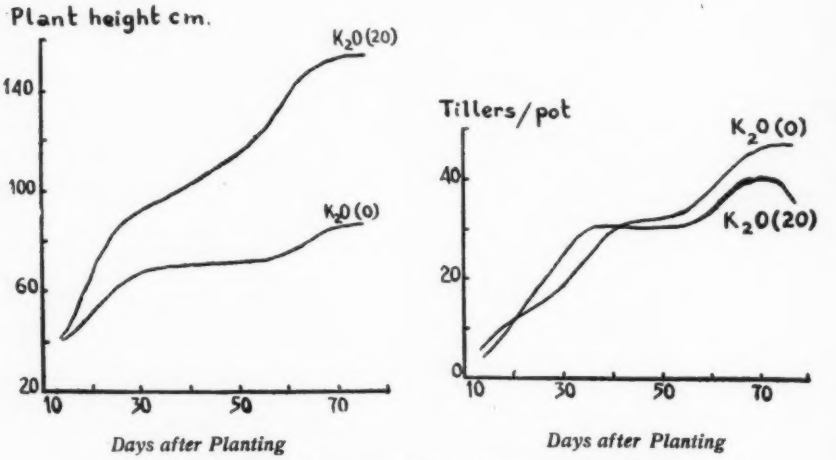

FIG. 3. Growth of rice plant at 0 and 20 p.p.m. K_2O.

(*b*) *Plant characters and dry matter weight at harvest.*—Influence of increasing potash levels on plant height, ear bearing tillers, root length and dry matter weight of ear, straw and root is shown in Table III. Plant height at harvest, at different potash levels, is seen to follow the same pattern as that at earlier stages of growth. Number of ear-bearing tillers per pot, unlike total tillers, is, however, low at no potash level. At potash levels from 5–200 p.p.m., there is very little change in ear-bearing tillers per pot. Root length of no potash plants, unlike that of no phosphate plants, is shorter than that at higher potash levels.

Total dry matter weight of plant in potash series is seen to increase with increase in potash level up to 200 p.p.m. Ear weight, however, increases sharply

TABLE III

Potash series—Growth characters and dry matter weight at harvest

Levels of potash p.p.m.	0	5	10	20	40	60	100	150	200
Plant height (cm.)	86	126	142	154	155	156	160	159	153
Root length (cm.)	29	41	42	39	40	39	44	39	37
Ear number/pot	18·0	43·5	42·0	40·0	37·0	41·5	47·0	38·0	43·5
Dry matter weight (gm./pot)									
Ear	9	49	60	78	79	80	80	87	89
Straw	27	73	85	84	86	87	90	95	92
Root	5	11	12	12	17	15	15	15	15
Total	41	133	157	174	182	182	185	197	196

only up to 20 p.p.m. and later, there is very little change up to 100 p.p.m. after which it again increases slightly. Straw weight, on the other hand, increases sharply only up to 10 p.p.m. potash and the increase at higher levels is not so marked.

(*c*) *Mineral content of plant at harvest.*—Potash content of ear, straw and root at increasing potash levels is shown in Fig. 4. It is seen that potash

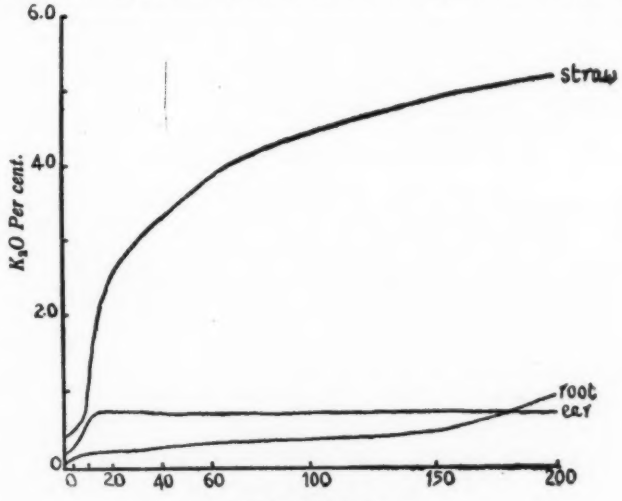

FIG. 4. Influence of potash level on K_2O content of rice plant.

content of ear increases upto 10 p.p.m. potash only and remains practically the same at higher levels of potash supply. Potash content of straw, on the other hand, increases with increasing supply of potash up to 200 p.p.m. and the increase is very sharp at 20 p.p.m. potash and upward.

Mineral content of a composite sample of plant is given in Table IV.

TABLE IV

Potash series—Nutrient content of rice plant at harvest

Levels of potash p.p.m.	N %	P_2O_5 %	K_2O %	CaO %	MgO %	SiO_2 %
0	2·82	1·90	0·34	0·75	0·41	6·20
10	1·50	0·94	0·75	0·69	0·30	3·45
40	1·40	0·72	1·95	0·52	0·20	2·55
100	1·20	0·72	2·45	0·50	0·19	2·40
200	1·14	0·60	2·90	0·44	0·15	2·40

Results show that increase in potash content of plant is accompanied by decrease in nitrogen and phosphate content. No potash plant shows the highest nitrogen and phosphate content and these drop sharply with potash supply up to 10 p.p.m. CaO, MgO and SiO_2 content of plant is also seen to decrease with increase in potash content.

DISCUSSION

Results presented show the influence of increasing levels of phosphate and potash in culture solution, on growth, yield and nutrient uptake by rice plant. Height, tillering and grain yield are all adversely affected at deficiency level of these nutrients. Figure 5 shows relationship between phosphate content of plant and dry matter weight of ear and straw.

Phosphate uptake by plant in relation to production of grain and straw can be divided into four stages. The deficiency stage of phosphate uptake is from 0–5 p.p.m., where both yield and phosphate content increase with increase in phosphate supply. The luxury absorption stage of phosphate is from 10–40 p.p.m. During this stage, grain and straw yield are little affected but plant continues to accumulate more phosphate with increased phosphate

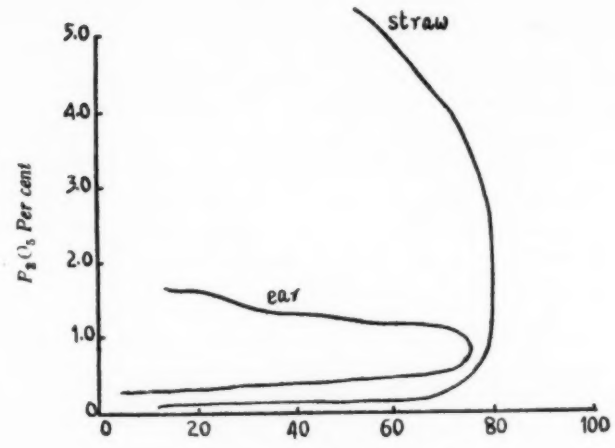

FIG. 5. Relationship between yield and P_2O_5 content of plant.

supply. The transition stage (5–10 p.p.m.) from deficiency to luxury absorption stage may be taken as the normal absorption stage for yield. The excess absorption stage for phosphate is above 40 p.p.m., where both grain and straw yields decrease, although phosphate content in all parts of the plant body increases, more markedly in straw. Results generally agree with the observations of Ishizuka and Tanaka (1951 *a*), for *japonica* variety of rice, except that the phosphate concentration needed for optimum yield of *japonica* variety is reported as 20–40 p.p.m., which is considerably higher than 5–10 p.p.m., found in the present study with an *indica* variety. There is also no mention of luxury absorption stage of phosphate in the *japonica* variety.

Phosphate deficiency symptoms, observed, were generally similar to those reported by Aiyer (1946) and Lockard (1956) for rice. Aiyer (1946) has, however, not observed any purplish colour of the dead leaves. Lockard (1956), from a series of sand culture studies, observed only little reddening sometimes on the older leaves, mostly localised in the tip areas. Phosphate deficient plants were also very low in K_2O and MgO content, while nitrogen and CaO content, though lower than that in the normal plant, was not seriously affected. Thus, potassium and magnesium deficiency may also accompany phosphate deficiency in rice plant. Silica content of phosphate deficient plants was very high, making the leaves stiff, straight and highly silicious to touch. Results regarding nitrogen, P_2O_5, K_2O, CaO and MgO

content of plant are in accord with the observations of Lockard (1956), on leaves of phosphate deficient plants.

Increased phosphate content in the harvest sample with increasing phosphate supply was seen to be associated with increased content of nitrogen, K_2O, CaO and MgO (Table II). Results are in accord with those reported by Aiyer (1946). This author has, however, reported negative correlation between phosphate and nitrogen content of straw and grain, which is contrary to the observations made in the present study. Increased nitrogen content in the rice plant, with increase in phosphate supply, however, has been observed by Sircar and Sen (1941).

With increasing phosphate supply, phosphate content of plant was seen to increase and this was more prominent in straw portion. Increase in phosphate content of the plant at phosphate levels of 60 p.p.m. and above was accompanied by decline in ear weight of the crop. Ishizuka and Tanaka (1951 *a*) have attributed the increase in phosphate content of the leaf at high phosphate levels, due to increase in inorganic phosphate content in the plant. It therefore appears that excess phosphate accumulation in the plant, at higher phosphate supply, makes the plant tissue inactive and causes physiological disturbance, pulling down yield considerably. The leaves of such high phosphate plants appeared partly drooping and withered, as if transpiring more water than leaves of normal plants. Russel (1950) has also suggested that excess phosphate over the amount required sometimes reduced crop yield.

In addition to above, nitrogen content of plants, grown at high phosphate levels of over 100 p.p.m., was seen to increase beyond the normal level. Sharp reduction in ear yield occurred in this range. Results of Tanaka *et al.* (1958 *a*) have shown that nitrogen content of plant at optimum level of nitrogen (40 p.p.m.) is about 1·3–1·5 per cent. These authors (Tanaka *et al.*, 1958 *b*) also reported that higher accumulation of nitrogen in the plant body resulted in disturbed nitrogen metabolism and low yield of grain. Reduction in the ear yield of crop at high phosphate levels in the present study may therefore be attributed both to disturbance in phosphate and nitrogen metabolism in the plant tissue.

The relationship between potash content and yield of grain with increasing potash supply is shown in Fig. 6. Three stages of potash absorption in relation to yield of grain and straw are observed. The deficiency stage of potash uptake is from 0–10 p.p.m, where ear yield and potash content of plant increase with increase in potash supply. The luxury absorption stage for potash is from 20–200 p.p.m., where both ear and straw yield are little

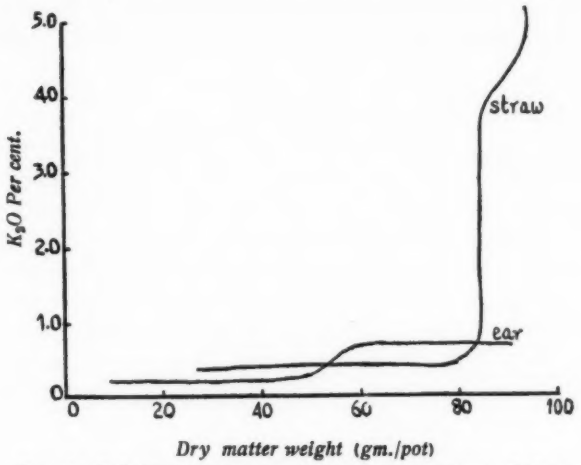

FIG. 6. Relationship between yield and K_2O content of plant.

affected, but potash content of plant, particularly of straw, increases with increase in potash supply. The transition from deficient to luxury absorption stage, from 10–20 p.p.m., may be taken as normal absorption stage for crop yield. These findings are in accord with those reported by Ishizuka and Tanaka (1951 *b*), for a *japonica* variety of rice. Unlike phosphate (in the present study) and nitrogen, reported earlier (Tanaka *et al.*, 1958 *a*), there is no evidence of excess absorption stage of potash even at 200 p.p.m. Results therefore show that in rice plant, build up of potash content at higher levels of potash is easily attained without any adverse or beneficial effect on plant growth.

Potassium deficiency in rice, unlike phosphate deficiency, does not result in very much reduced tillering capacity of the plant (Tables I and III). Potash deficiency symptoms, observed at lower levels of potash supply, are generally similar to those reported by Aiyer (1948) and Sircar and Dutta (1957), for rice. Lockard (1956), from a series of sand culture studies, has, however, reported that in practice, it was often difficult to distinguish potassium deficiency from phosphate deficiency. In our trials in solution culture, however, potash deficiency was easily distinguishable from phosphate deficiency. The main distinguishing features were the highly reduced tillering capacity due to phosphate deficiency and appearance of purplish red colour in the old and dead leaves of phosphate deficient plants. Potash deficient plants, on the other hand, had general brown spotting of leaves, with decaying along leaf tips. There was absence of yellow or purplish colour in old and dead leaves,

generally associated with nitrogen and phosphate deficiencies respectively. Colour of old and decayed leaves of potash deficient plants tended to be dirty yellow.

Potash deficient plants had higher content of both nitrogen and phosphate. Results are in agreement with observations of Aiyer (1948), Noguchi and Sugawara (1952) and Lockard (1956), for rice. Sircar and Dutta (1957), however, did not find increase of total nitrogen content in potash deficient rice plants, but observed increase in soluble nitrogen fraction, comprising of ammonia, amine, amide and nitrate nitrogen. Instances of potash deficiency, resulting in higher accumulation of soluble nitrogen fraction in the plant body are also reported by Richard and Templeman (1936) in case of barley plant and by Wall (1940) in the case of tomato plant. It has been suggested by these authors that this accumulation leads to the breakdown of nitrogen metabolism in the plant body.

As regards phosphate content of potash deficient plants, it is seen that, while potash deficiency results in high accumulation of phosphate in the plant body, phosphate deficiency, on the other hand, is accompanied with low absorption of potash.

Potash deficiency in the rice plant is also accompanied with high content of MgO, CaO and SiO_2 and with increased absorption of potash, there is progressive reduction in the absorption of MgO and CaO. Silica content also decreases up to 40 p.p.m. potash. This relationship between potash deficiency and MgO and CaO has also been observed by Aiyer (1948) in rice and Hoagland and Martin (1933) and Walsh and Clark (1945) in tomato plants. Lockard (1956) has, however, reported increase in CaO content but no influence on MgO content with potash deficiency in rice. Increasing absorption of potash at high potash supply, however, does not decrease CaO and MgO content of rice plant to such a low level as to show any potash induced magnesium deficiency, observed in horticulture and market garden crops (Walsh and O'Donohue, 1945).

SUMMARY

Increasing levels of phosphate and potash in the culture solution were seen to produce a series of changes in growth and nutrient uptake by rice plant. Optimum growth and yield of grain was obtained at 5–10 p.p.m. phosphate and 20 p.p.m. of potash respectively. Deficiency of both these nutrients occurred at low levels, where grain yield was pulled down. Higher levels of phosphate over 40 p.p.m. were seen to be toxic and reduced grain

yield. No adverse effect due to high supply of potash was observed and plants appeared unaffected by luxury consumption of potash.

Phosphate deficiency was seen to result in stunted plant growth with reduced tillering capacity. Formation of ear heads was poor and grain yield was low. Leaves were narrow, stiff, straight and bluish green in colour. Lower part of the stem was coloured with purplish streaks and lower leaves started drying from tip downwards. The colour of dead leaves was purplish red. Primary roots of phosphate deficient plants were longer and secondary roots were scanty and colour of roots was rust brown. Phosphate deficient plants had a very low content of K_2O and MgO. Silica content of phosphate deficient plants was very high, making the plants stiff and straight. Phosphate toxicity occurred at higher levels of 100 p.p.m. phosphate and above and resulted in high accumulation of nitrogen and phosphate in the plant tissue.

Potassium deficient plants were also stunted in growth but tillering was not so adversely affected as in phosphate deficient plants. Upper leaves of potash deficient plants were deep green, soft, small and wavy in appearance. Lower leaves were characterised by development of dark brown spots with drying at the tips. Colour of old and decayed leaves was dirty yellow. Root development was poor and stunted. Primary roots were shallow and there was scanty formation of secondary roots. Potash deficient plants were characterised by high nitrogen and phosphate content and percentage of these in the plant decreased with increase in potash content. With increase in potash content of plant at higher levels of potash, CaO and MgO content decreased but deficiency level of these was not reached even at luxury consumption level of potash.

Authors are indebted to Mr. R. L. M. Ghose, Director, Central Rice Research Institute, Cuttack, for his suggestions and improvement in the text of the paper.

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FURTHER CONTRIBUTIONS TO OUR KNOWLEDGE OF *ISŒTES SAMPATHKUMARANI* RAO, L. N.

Part II. Some Stages in the Megasporogenesis and Germination

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Introduction

The morphology and anatomy of the vegetative organs of the interesting species of *Isætes*, *I. sampathkumarani* has been recently studied (Sharma, 1958, pp. 210–24). The present paper deals with the reproductive parts of the same species as observed from continued investigation of the same material and also from fresh collections. As was stated in the earlier paper, the plant bears normally sporophylls with a few sterile leaves generally placed on the periphery. It was found that the first few leaves on a young rhizomorph are generally sterile, and that in these the ventral lobe of the velum is not generally developed. In older plants, almost all the sporophylls were found to be megasporophylls. An intriguing feature so far as I have observed is the absence of the microsporophylls. In fact Prof. L. N. Rao (1944, pp. 286–87) made a similar observation also. I have carefully examined plants collected at different parts of the year, but so far, I have not been able to recognise a single clear microsporophyll with microspores. Nor have I seen in the material any scattered spores that could be recognised as microspores. It is unlikely that this species does not at all produce microsporophylls. But a greater likelihood is that these microsporophylls are produced perhaps seasonally as in some species of *Isætes* (Pfeiffer, 1922, p. 88) and probably the duration of this season is too short to be noticeable. It might be pointed out here that in *I. nigritiana* also (Pfeiffer, *loc. cit.*) no microsporophylls have been noticed. Naturally, in the absence of microsporophylls, it was possible to study only the megasporophylls and some aspects of megasporogenesis. All the stages of megasporogenesis were not available. Only such stages as could be clearly seen in megasporogenesis and germination of spores have been figured and described. Older stages and details regarding the gametophytic development will be elucidated after further examination of the material which is under progress.

Material and Methods

Most of the material was fixed in 90% formal acetic alcohol but afterwards transferred to 90% alcohol. Some material which was brought in a living condition to this place was fixed in pure 90% alcohol as well as 90% formal acetic alcohol. Studies were made with the help of serial, microtome and hand sections.

Sections were cut of young and old entire plants so that as many stages as possible of development of the sporangium and spores could be seen in a single section. Mostly the sections were cut 8 μ in thickness but few were also cut 10–12 μ.

For staining and embedding purposes the technique recommended by Professor Maheshwari (1933, pp. 129–30; 1939, pp. 257–81) was followed. Iron-alum-hæmatoxylin stain was mostly used. Orange G. in clove oil also gave good results.

The megaspore offered a little difficulty in cutting. This was overcome by dissolving the sileceous outer coating of the spores by treating them with dilute commercial hydrofluoric acid for a period of at least 24 hours and then washing them in water and dehydrating as usual.

Earlier Work on Megasporangial Development and Megasporogenesis

Earlier workers like Hofmeister (1862), Goebel (1881, 1884, 1887), Farmer (1890–91), Bower (1897 and 1908) and Wilson Smith (1900), etc., working on different aspects of various species of *Isætes* have discussed the origin of the sporangium in detail. But recently no work seems to have been published on this particular aspect, specially on any Indian species of *Isætes*. A detailed cytological work, however, was published in 1933 by Ekambaram and Venkatanathan, wherein they have briefly touched the development of the sporangium in *Isætes coromandelina* L. They have also given a good summary of the earlier work and opinions on the subject.

There are two different views expressed about the initiation of the sporangium. The first view enunciated by Hofmeister is that the sporangium arises from a single superficial cell—which divides into two, the upper one forming the velum initial and the lower one after division in all three directions forming the sporangium (Hofmeister, 1862, p. 364).

Bower supported this superficial origin of the sporangium but corrected Hofmeister to the extent of saying that the sporangium arises from a transverse group of superficial cells (Bower, 1897 and 1908, p. 319) and not a single cell.

Wilson Smith too from his study of *I. echinospora* and *I. engelmanni* supported Bower. But he also pointed out that the later stages of sporangial development differed in several respects from those observed by Bower (Wilson Smith, 1900, p. 235).

Ekambaram and Venkatanathan (1933, p. 201) also subscribe to the theory of the superficial origin of the sporangium from their study of *I. coromandelina*.

Hegelmaier (1874) and Tschistiakoff (1873, *see* Ekambaram and Venkatanathan, *loc. cit.*, p. 192), on the other hand, thought that the sporangium is developed from a group of deep-seated initials. They also were of the

opinion that the wall of the sporangium is present from the beginning (" von Anfang an gesondert ", *see* Wilson Smith, 1900, p. 239).

Goebel (1887, p. 293) also supported this hypodermal origin of the sporangium from his observations of *I. lacustris*.

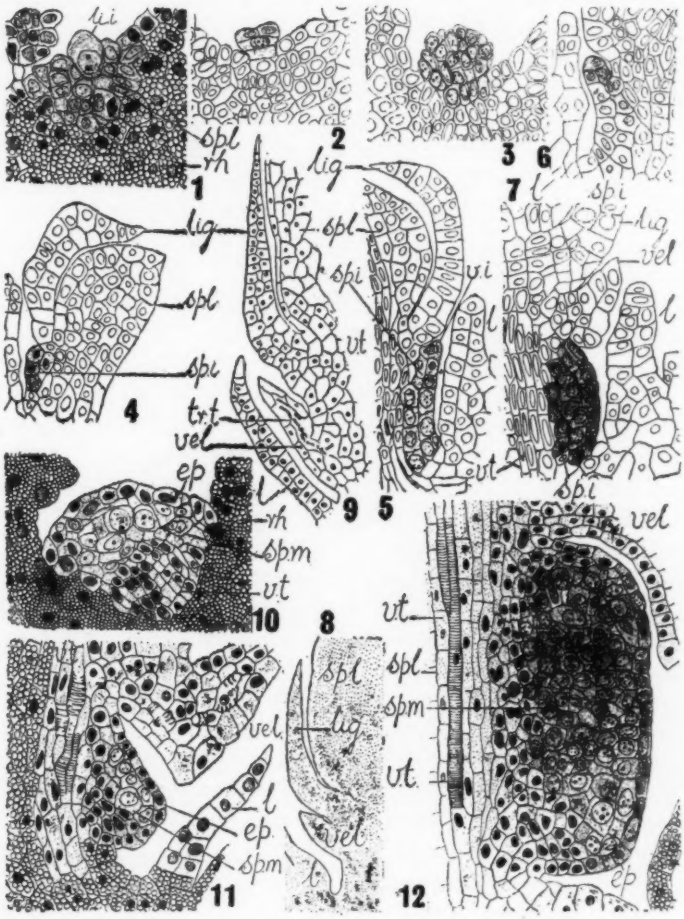
It is not necessary to describe here in detail all the views on the subject. A good review of the various opinions is given by Ekambaram and Venkatanathan (*loc. cit.*, pp. 192–94) and Wilson Smith (*loc. cit.*, pp. 234–35).

So far as *I. sampathkumarani* is concerned, it seems to have a superficial origin of the sporangium and appears to resemble in general features of sporangial development, the species *I. echinospora* and *I. engelmanni*. In its cytological features it resembles *I. coromandelina* to a great extent. The different developmental stages will now be described.

MEGASPOROPHYLL

Ligule.—The ligule is initiated by a single cell which is distinguished from its neighbours by its large size and rich protoplasmic contents as shown in transverse sections of the leaf in Fig. 1 (*li.i.*). Later this cell divides transversely into a 3-celled filament, the end cell of which now divides longitudinally as in Fig. 2. These cells further divide to form a mass of cells as in Fig. 3. Subsequent transverse and longitudinal divisions add to the form of the ligule. Similar stages of development of the ligule are figured by Wilson Smith (1900, Figs. 12–19) in *I. engelmanni* and *I. echinospora*. The other details about the ligule structure have already been described (Sharma, 1958, pp. 216–18).

Velum.—It is found that the velum develops on the sporophyll almost at the same time as the sporangium. A row of large deeply staining superficial cells distinguish themselves as the sporangium initials (*sp.i.*, Figs. 4 and 5). The upper end cell (*v.i.*, Fig. 5) divides into a group of three cells (Fig. 6). These constitute the beginning of the velum. It is found that the velum develops more rapidly than the sporangium (*vel.*, Fig. 7 and Photo 1). It has also been found that in sterile leaves the velum (*vel.*) does not produce the lower lobe (Fig. 8). The velum in its younger stages is 3 cells thick with a central row of tracheidal cells (*tr.t.*, Fig. 9). The occurrence of these tracheidal cells is also reported by different authors in different species, as for example in *I. hystrix* (Scott and Hill, 1900, p. 434), in *I. echinospora* and *I. engelmanni* (Wilson Smith, 1900, p. 242), *I. lacustris* (Mclean and Ivemey-Cook, 1951, p. 600) and in *I. coromandelina* (Ekambaram and Venkatanathan, 1933, pp. 199–201). In *I. hystrix*, *I. echinospora*, *I. engelmanni* and *I. lacustris* there is no connection between the tracheidal tissue and the vascular supply



FIGS. 1–12.

of the leaf, while according to Ekambaram and Venkatanathan (*loc. cit.*, p. 201) there is definitely such a connection in *I. coromandelina*. In *I. sampathkumarani* it was not possible to see any connection between the vascular supply of the leaf and the tracheidal cells of the velum (Sharma, 1958, p. 218).

Megasporangium.—The megasporangium as stated above is initiated by a row of superficial cells (*sp.i.*, Fig. 4). These cells divide by periclinal walls into a double row of cells (*sp.i.*, Fig. 5). Subsequent rapid divisions of these

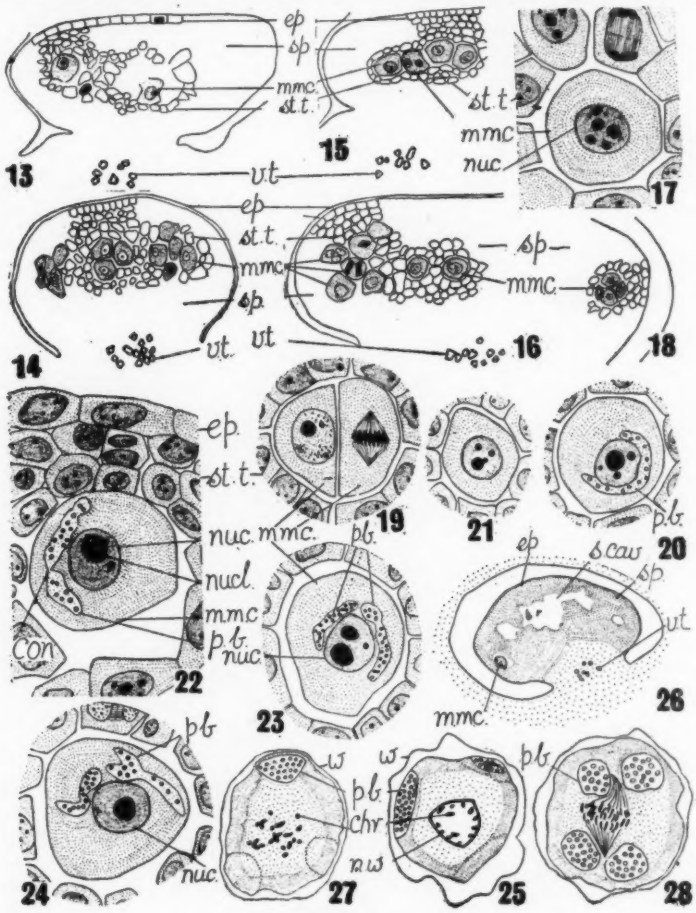
cells build up a tissue (Fig. 7) which is later delimited from the surface by a row of distinctive cells forming the epidermis (*ep.*, Figs. 10–12). As Wilson Smith (*loc. cit.*) has pointed out in *I. echinospora* and *I. engelmanni* the archesporium and the epidermis are differentiated ultimately from the same tissue, derived by the periclinal division of the original row of initials. The outermost layer of this tissue forms the wall. This is definitely a pteriodophytic character according to him. In *I. sampathkumarani* also, the same arrangement holds good. In all the three above-mentioned species the sporangium can be regarded as " growing as a unit and not as a number of individual segments " (Wilson Smith, *loc. cit.*, p. 241). Later these cells, bounded on the outer side by the epidermis and on the inner by the cells of the sporophyll, constitute the final archesporial mass. They divide and build up the homogeneous tissue of the megasporangium (*sp.m.*, Fig. 12). The megaspore mother cells are differentiated in this tissue at a later stage. They may be in the form of isolated cells (*m.m.c.*, Fig. 13) or in the form of groups (Fig. 14) or in rows (Fig. 15) or in both groups and rows (Fig. 16). These rows or groups of cells both in the vertical and horizontal planes are not continuous but are discontinuous. There is thus no continuous columnar megaspore mother cell tissue. The megaspore mother cells in young stages are roughly hexagonal in form with dense protoplasmic contents and a big nucleus (*nuc.*, Fig. 17). The number of nucleoli vary from 2–7 in each nucleus (Fig. 17). A large number of nucleoli are also known in *I. echinospora* and *I. engelmanni* (Wilson Smith, p. 252). As soon as the megaspore mother cells (*m.m.c.*, Figs. 13–16) are formed they seem to break off from the surrounding cells (*st.t.*) and get separated from them; a feature that is clearly observable in Figs. 13–16. This degeneration of the surrounding tissue gradually creates the empty spaces or the cavities of the megasporangium. The tissue, that does not degenerate like this, ultimately constitutes the trabeculæ and the tapetum. The same phenomenon happens in other species of *Isætes* like *I. coromandelina* (Ekambaram and Venkatanathan, *loc. cit.*, p. 205), *I. echinospora* and *I. engelmanni* (Wilson Smith, *loc. cit.*, p. 255). It is found that the vascular supply of the megasporophyll becomes elaborated as it gets older. In the very young stages the megasporophyll does not show much of vascular tissue.

It is also found that not all the megaspore mother cells ultimately produce megaspores. A few of these megaspore mother cells (*m.m.c.*, Figs. 16, 18 and 19) may divide quite early in their life and produce a number of thin-walled cells which add to the amount of sterile tissue in the sporangium. This has been observed in *I. coromandelina* (Ekambaram and Venkatanathan, 1933, pp. 202–03) and also in *I. echinospora* and *I. engelmanni* (Wilson Smith,

1900, p. 253). It thus appears that for some reason or other a few of the megaspore mother cells do not bring themselves up to that efficiency of producing megaspores, but degenerate into sterile cells. These cells may divide repeatedly to build up the sterile tissue (Fig. 17). The megaspore mother cell determination, according to Wilson Smith (*loc. cit.*, p. 254), is conferred more " by advantageous environment rather than morphological position".

Cytology

A fully developed megaspore mother cell (*m.m.c.*) is ellipsoidal in outline, with a dense, richly granular cytoplasm and a fairly large-sized nucleus (*nuc.*,



Figs. 13–28.

Fig. 17). Fat globules are not seen probably because the material was not killed in osmic acid. The nucleus is situated in the centre of the megaspore mother cell (Fig. 17) or slightly towards one side (Fig. 20). It has got a faintly staining reticulum (Photo 2), and some nucleoli whose number varies from 2–7 (Figs. 17 and 19–22). They stain deeply with iron-alum-hæmatoxylin. Nucleolar budding (Fig. 21) like that found in *I. coromandelina* (Ekambaram and Venkatanathan, 1933, p. 207) has also been observed but rather rarely.

Heterotypic prophase.—The first visible change in the cytoplasm of the megaspore mother cells is the appearance of a colourless starch studded body very close to the nucleus. Afterwards this body assumes the form of a crescent closely applied round the nucleus (*p.b.*, Fig. 20) as in *I. coromandelina* also. There seems to be a connection between this body and the reduction division of the megaspore mother cell. It is therefore not justifiable (Ekambaram and Venkatanathan, 1933, p. 206) to call this body as a mere starch clump as some people have done. These polar bodies are evidently the " mantle of starch grains " referred to by Fitting (1900, *see* Verdoorn, 1938, p. 226) in his study of *Isætes* and *Selaginella* species. These ' mantles ' also behave in the same way as these polar bodies under discussion. Their nature is probably like a centrosome. The name " Polar bodies " adopted by Ekambaram and Venkatanathan has been used here.

A subsequent change in the polar body is its constriction (*con.*) near about the middle (Fig. 22) to form two polar bodies which occupy positions opposite to each other (Fig. 23). In addition to the starch grains several other granules are present in the polar bodies. This is followed by a second constriction of both the polar bodies (Fig. 24) resulting in four bodies which gradually assume an elliptic or spherical form. These four polar bodies (*p.b.*) occupy the corners of a tetrahedron (Fig. 25) as in *I. coromandelina* (Ekambaram and Venkatanathan, 1933, p. 208). The cytoplasm surrounding these polar bodies is denser towards the cell-wall (Fig. 25). The polar bodies do not show any further change in size, shape or number. They are fairly constant. At this stage the megaspore mother cells come to lie freely in the central space of the sporangium (*s.cav.*) formed by the dissolution of the surrounding tissue (Fig. 26).

Diakinesis.—The next important stage observed was the replacement of the reticulum of the nucleus by short thick chromosomes (*see* Fig. 25). Simultaneous with these changes, the nucleus assumes a roughly triangular outline (Fig. 25) in sectional view, with the polar bodies (*p.b.*) opposite the angles. The formation of spindle fibres is not seen clearly, probably due to

defective fixation of material. The nuclear membrane (*n.w.*, seen in Fig. 25) also disappears gradually and a number of short rod-like chromosomes (*chr.*, Fig. 27) come to lie in the centre. They seem to be transversely or obliquely cut as in Fig. 27 and are better seen in Fig. 28 and Photo 3, which seem to show the heterotypic metaphase. The spindles become bipolar. The four polar bodies which originally occupied the four corners of the tetrahedral body arrange themselves in two pairs forming the two poles of the heterotypic metaphasic spindle (Fig. 28) as is also reported in *I. coromandelina* (Ekambaram and Venkatanathan, 1933, p. 209).

In Fig. 28 there can be noticed the absence of the nuclear membrane; the presence of four polar bodies, two at each pole and the spindle originating at each pole between a pair of polar bodies. The chromosomes are all placed in the equatorial region and a careful examination of the section shows that the chromosomes are arranged as though in pairs. There seems to be no doubt that this represents the heterotypic metaphase.

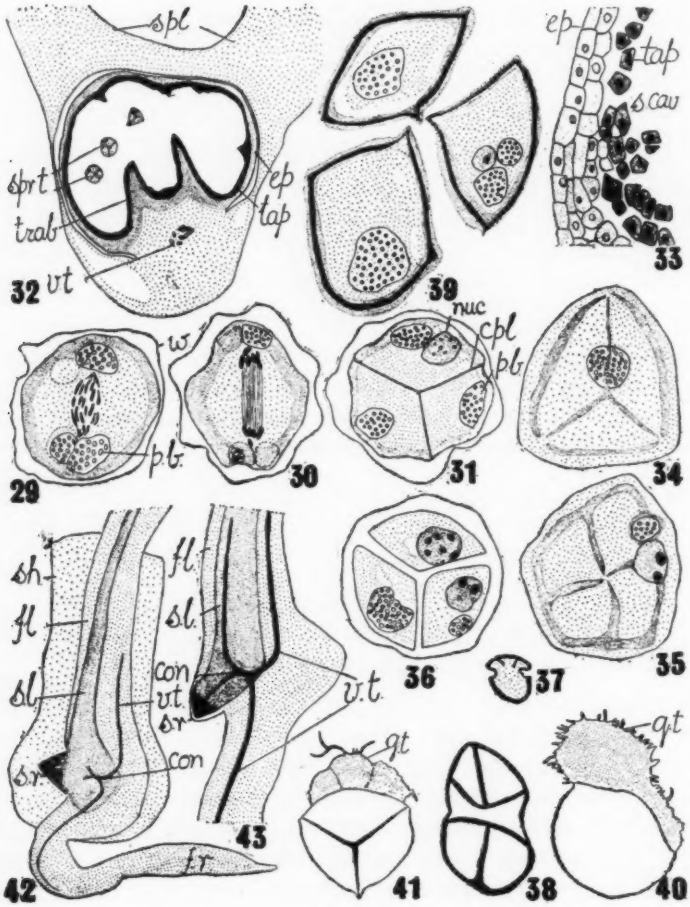
It was difficult to count the chromosomes because of their large number. Recently Abraham and Ninan (1958, pp. 60–61) and Ninan (1958, pp. 93–103) have reported the presence of 66 chromosomes in the homotypic division of the root-tip cells of *Isœtes sampathkumarani.*

Anaphase and Telophase.—Two stages of anaphase are also recognizable. In one, some of the chromosomes are moving from the equatorial plate while a few are still there (Fig. 29).

A late anaphase stage is seen in Fig. 30. The chromosomes are collected at the two poles and the spindle fibres are also seen very clearly. Two pairs of polar bodies are seen, one pair at each pole.

The telophasic stages and the earlier stages of homotypic division have not been found yet. But some of the preparations show the cell plate (*c.pl.*) between the four daughter nuclei along the equatorial plane in the spore mother cell (Photo 4, and Fig. 31). In each of the new spores thus defined, a single polar body and a single nucleus can be located. Figure 32 shows such spore mother cells, in early spore tetrad (*spr.t.*) stages, lying inside the sporangial cavity. The tapetal cells (*tap.*) lining the sporangial cavity (Figs. 32 and 33) can also be seen. The tapetum generally appears late, at about the diakinesis stage of the megaspore development. It is differentiated from the vegetative cells that line the inner walls of the sporangial cavities. It appears therefore that the tapetum is derived from the sterile cells. These may belong to the mass of vegetative cells of the sporangium or the cells rendered sterile by the conversion of some divided megaspore mother cells.

The tapetum generally decreases as the megaspores develop further and ultimately disappears. In the very young and very old sporangia no tapetum is present. The origin and development of the tapetum in *I. sampathkumarani* is in general respects similar to that of *I. coromandelina*.



FIGS. 29–43.

The trabeculæ (*trab.*, Fig. 32), seen as plates of tissue projecting into the sporangial cavity, are merely the tissue left without being disorganised. This is the case in *I. coromandelina* also. An interesting variation is that although the spores are grouped together generally in a tetrahedral form (Figs. 31,

32 and 34), they may sometime be formed in bilateral groups as shown in Figs. 32 and 35. This is known also in *I. engelmanni* and *I. echinospora*. The walls separating the spores from each other are finely stratified. A little later splitting takes place all along these separating walls (Fig. 36) and the spores are gradually separated from each other but are still grouped together inside the thick megaspore mother cell wall (*w.*, Fig. 36). The large nucleus may contain even at this stage a varying number of nucleoli but generally a single polar body (Fig. 36). Sometimes the spores may not separate but remain attached in pairs not only in younger stages (Fig. 37) but also in older stages (Fig. 38). Figure 39 shows the spores separated from each other and the spore wall differentiated into three layers. The spores seen in Fig. 39 show also the sharp ridge formed along the triradiate mark. At this stage some of the spores may also show a large polar body which may even be divided. The protoplasm also becomes highly vacuolated.

Spores

The fully developed spore measures 443 μ, shows no polar body, has a thick wall (Photo 5), which can be differentiated into an outer glassy or colourless layer, which is uneven, brittle, stains poorly and is known as the epispore (*epi.*, Photo V) or perinium. This layer is according to Farmer (1890–91, p. 54) derived from the plasma of the sporangium and the disorganising tapetum. The next inner layer known as the exospore (*exo.*, Photo 5) stains easily with orange G. This layer can be differentiated into an outer stratified (*o.l.*) and an inner homogeneous layer (*i.l.*). The innermost endospore appears to be of cellulose nature and of varying thickness. Farmer (*loc. cit.*) has discussed fully the nature and origin of these layers. I agree with his observations. The interior of the spore contains a large nucleus and no polar bodies but a large number of starch grains, oil drops and vacuolated cytoplasm can be made out. All ripe spores are dark in colour and show the same type of wall structure. It is however found that there are also spores smaller than the ones described above (Photo 6).

All the spores of a tetrad are of the same size in the early stages. A differentiation between the above two kinds of spores manifests itself almost immediately after the release from the tetrad stage. The differences become more pronounced as the sporangium grows older. Since the wall structure in both cases is similar, it is considered that this difference in size is brought about purely by retarded growth in the smaller variety of spores. Curiously enough both these kinds of spores are always found in the same sporangium and in equal proportions, a fact which was confirmed by repeatedly teasing sporangia and examining their contents. These smaller spores measure 287 μ

and have poorer protoplasmic contents, although they have a nucleus with four nucleoli. It may be that we are dealing here with a case of dimorphism of spores as is recorded in *I. welwitschii* and *I. æquinoctialis* (Pfeiffer, 1922, pp. 106 and 107) and *I. dixitii* (Shende, 1942). It might be pointed out here that germination stages show that both kinds of spores germinate and produce what is evidently the gametophytic tissue (Photo 7).

All spores when moist are darkish in colour and become white when dry as observed by Professor L. N. Rao (1944, p. 287). The megaspores are 287 μ and 443 μ and as observed by Professor L. N. Rao have "prominent commissural ridges, the upper segment marked with reticulate branched ridges".

Seedlings

Attempts were made to germinate megaspores in tap-water, Knop's solution, on damp mud, etc., but without success. However, the mud in which fresh plants were growing in nature was carefully examined and several stages of germination were found. Earthworms according to Duthie (1929, pp. 411–12) are responsible for the dissemination of megaspores in three South African species of *Isætes*. But in the mud in which these megaspores and young plants were growing there were no earthworms at all. It therefore seems unlikely that the spores are disseminated with the help of earthworms in this species. The earliest stage of germination observed was the presence of a small knob-like growth coming out from both kinds of megaspores referred to above (Figs. 40, 41 and Photo 7). The spore generally cracks along the triradiate mark and the knob-like white tissue may burst through this in one, two or even three, masses like the gametophytic mounds of some species of *Selaginella*, *S. galeotti* (Eames, 1936, p. 41). In many cases these tissues are found to come out not from the triradiate split which is intact but from the other side. The wall of the spore becomes soft and is pushed out allowing the gametophyte to come out (*g.t.*, Figs. 40 and 41 and Photos 8 and 9). This tissue represents evidently the gametophytic lobes because they bear rhizoids also (Figs. 40 and 41). But unfortunately no archegonia have so far been observed in this tissue, which is made up of small, elongated, thin-walled cells (Photo 10) arranged in vertical rows, like plates of tissue.

It has not been possible to elucidate any stages of microsporogenesis or fertilization as microsporophylls have not been found so far. However, some early stages of the seedlings were secured. It is found that the leaf develops earlier than the root. In some of the younger seedlings only a

single leaf and a single root are found (Photo 11). Later (Fig. 42) the second leaf (*s.l.*) and the second root (*s.r.*) are formed, the former bursting through the gametophytic sheath (*sh.*). Sometimes the remnants of the megaspore wall may also be seen (Photo 11). In fact a certain amount of gametophytic tissue remains also attached to the seedling and in one of them was found what looked like an old archegonium. But since this was not fully and satisfactorily preserved no figure is given. In the seedling of two to three-leaved stage a rhizomorph appears to differentiate.

At the one-leaved stage the vascular supply of both the root and the leaf is a single continuous strand with a constriction (*con.*) at the level of the hypocotyl (Fig. 42). The vascular supply of the second leaf branches off from this junction (Fig. 43). Subsequent leaf traces also come off from here and at about a three-leaved stage this junction becomes a little thicker and develops starch-laden cells round about it. This may be interpreted as the earliest stage of the development of the rhizomorph. These first formed vascular traces contain mostly spiral and annular tracheids. No clear phloem tissue is however recognizable in the seedling stage. The spore wall is still attached to the young seedling till its three-leaved stage and then drops off (Photo 12). The roots now develop rapidly and dichotomise (Photo 12, extreme right).

The adult rhizomorph of *I. sampathkumarani* (Sharma, 1958, p. 212), although normally two-lobed, occasionally shows three to four lobes. Similarly, the normally three-lobed rhizomorph of *I. coromandelina* often shows four or five lobes. In *I. muelleri* (Baker, 1887, p. 127) nine lobes are met with normally. These varying numbers of lobes in the same species as well as in different species leads one to think that the normal number of lobes in any species is a derived condition. In the ancestral condition a large number of lobes—perhaps stunted branches—were probably prevalent. If this is accepted, then any two adjacent lobes would constitute a dichotomy of a branch and the varying number of lobes in the rhizomorph of *Isœtes* could be interpreted in terms of varying degrees of dichotomy.

Schoute (1938, Chapter I, pp. 1–64) discussed morphologically controversial plant organs and on the basis of morphological and anatomical studies he thinks that the rhizomorph in *Isœtes* " is only peculiar in its constantly *tearing* furrows, a feature connected with the formation of local humps of secondary tissue, the lobes". In other words according to him, the lobes are nothing more than humps of tissue which are the result of localised secondary activity, the position of these humps being determined by the phyllotaxis of the young plant. The rhizomorph stands no comparison with the rhizophore of *Selaginella* or the *Stigmarian* axis according to him.

A chart describing the important characteristics of Indian species of Isœtes

Name of species	*Isœtes coromandelina* L. Ekambaram and Venkatanathan (1933)	*Isœtes sampathkumarani* Rao, L. N. Rao (1944); Sharma (1958)	*Isœtes sahyadrii* Mahabale Mahabale (1938)	*Isœtes dixitii* Shende Shende (1942)
Stature of plant	Plants up to 53 cm. long	10 to 11 cm. long plants	7–20 cm. long plants	
Habit ..	"Amphibious, fringing the water edges of lakes, ponds or pools. Never found in deep water, a portion of the leaves being above the water-level"	Amphibious "along water margins in shallow depressions in granite rocks. Associated with species of *Eriocaulon*, *Myriophyllum* and *Illysanthes parviflora*"	"Occurring at the close of monsoons on the margins of ponds in the midst of what may be called *Ammania-Eriocaulon* association"	..
Rhizomorph ..	Generally 3-lobed, often 4 or 5-lobed	Deeply 2-lobed; often 3 to 4-lobed	3-lobed	3-lobed
Sporophyll ..	Leaves 15–16, up to 50 cm. long. Slender with a lower half flattened into membranous wings, spreading, stomata present, peripheral strands present	Leaves 3–16 spiral up to 10 cm. long, slender, with a membranous margin at the base and gradually tapering at the apex, spreading, stomata present only on the upper half, air-chambers 4, peripheral strands absent	"Leaves 7–15, 7–20 cm. long, slender, cylindrical and dark-green, stomata numerous on the upper part of the leaf, air-chambers 4, peripheral strands none"	..
Presence or absence of vegetative leaves	Vegetative leaves occur on the outer side	Vegetative leaves few and occur only on the periphery	..	..
Velum ..	Only the upper lobe of velum present and the lower lobe is not formed at all	Partial (incomplete)	Complete, leaving only a slit-like aperture at the base	Rudimentary and covering half or even less of the sporangium
Ligule ..	Ligule small in comparison to big-sized sporangium, slightly triangular or elliptic	Ligule may be triangular, cordate broadly elliptic, etc.	Cordate, slightly lobed on the flanks	..

Megasporangium ..	Circular, about 5 mm. in diameter	Oblong, nearly 2 mm. long	2 mm. more or less round	..
Megaspores ..	"480–540 μ, megaspores white when dry, grey when moist, somewhat flattened, marked with numerous large tubercles, closely arranged and occasionally extended ridges into very short rounded ridges"	Dimorphic, small 287 μ, big 443 μ, have got prominent commissural ridges, the upper segment marked with reticulate branched ridges	"325–430 μ in diameter, cream-coloured when dry, dark-brown when wet, studded with large uneven tubercles particularly on the lower part, commissural ridges prominent"	Megaspores marked with round, non-confluent tubercles and dimorphic megaspores in the same megasporangium
Microsporangium	More or less equal to megasporangium	No microsporophylls found so far	..	..
Microspores ..	26–33 μ, chiefly 30 μ, smooth, red-brown or paler at maturity	..	30 μ in diameter marked with small spines	..
Chromosomes found in root-tips	According to Abraham and Ninan diploid number of chromosomes is 22 and a fragment (or B chromosome); according to Ekambaram and Venkatanathan haploid number is 16	Diploid. Root-tip chromosomes 66 (Abraham and Ninan)	..	..
Distrib ..	All over India	Found in Lalbagh Gardens of Bangalore (Mysore State) and also in Waltair (Madras State)	"The mountain lake on the tableland at Panchgani (Bombay Presidency) 4,500 ft. above the sea-level." "In ponds on the grassy tops of the Kalahattigiri, Bababudhangiri and other peaks of Sahyadris in the Mysore State"	Panchgani

Another possible explanation deserving consideration has been advanced by various other earlier workers (*see* Schoute, 1938, p. 58) and also Eames (1936, pp. 351–56). They regard that the Isœtalean rhizomorph is comparable with a Stigmarian subterranean axis.

Although no fresh morphological or anatomical evidence has been advanced, the view of Eames and others can be supported. *Isætes* is regarded as a highly telescoped strobilus or a plant like *Pleuromeia*. If the sub-aerial parts of considerable dimensions have undergone this reduction in size, there is no reason why the underground branching system, comparable to the rhizophore of a *Selaginella* or the axis of a *Stigmaria*, could not also be shortened to various degrees and the branches reduced to swollen " lobes".

It might be that as a concomitant of this abbreviation and swelling, the roots which were probably borne further off were developed on these stunted branches. The fact that the roots of *Isætes* are also anatomically similar to those of *Stigmaria* is another point to be noted (Eames, 1938, p. 354, Fig. 205).

These varying number of rhizomorph lobes, even in one and the same species of *Isætes*, interpreted in terms of indefinite dichotomy as discussed above, may be due to various degrees of suppression of a once widely prevalent dichotomy of appendageless subterranean axes. Whether these subterranean organs were similar to those of *Stigmaria* or were comparable to the rhizophore of a *Selaginella* still remains an unsolved problem.

The main features of the vegetative parts and the megasporophylls of *I. sampathkumarani* have been compared with corresponding features of other Indian species in the following table. Other points of morphological and taxonomical importance concerning the Indian species have also been incorporated in the table as far as they were available. I hope this will be of help to the student of pteridophytes in identifying the Indian species of *Isætes*. The table can be improved upon as more facts become available.

Summary

In the present paper the development of the ligule, velum and megasporogenesis of *Isætes sampathkumarani* Rao, L. N. are studied. The ligule is initiated by a single cell, while the velum and megasporangium have a common origin from a superficial row of cells. The terminal cell of this row divides and builds up the ligule, which grows more rapidly than the sporangium. The megasporangium grows as a single unit and not as a number of individual segments. The megaspore mother cells are sorted out of this

tissue. They may occur scattered, or connected in rows, or in groups. While most of them develop spore tetrads, others might divide and build up vegetative tissue. The spore mother cells separate from the surrounding tissue by the dissolution of the vegetative cells. The sterile tissue, that is not thus disorganised but remain behind, forms the tapetum and trabeculæ.

The heterotypic prophase, diakinesis, heterotypic metaphase, anaphase and early stages of the tetrad formation are studied and described. An interesting feature of this species is the presence of dimorphic megaspores.

The sporangial development is similar to that of *Isœtes echinospora* and *I. engelmanni*. The few cytological observations made resemble those of *I. coromandelina*.

Microsporophylls or even scattered microspores have not yet been found. Still, germinating megaspores show plate-like gametophytic tissue bearing rhizoids, growing out from the spores. The vascular structure of the young seedling has also been studied. A table of the Indian species of *Isœtes*, with their distinguishing characters so far known, has been included.

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This work has been carried out during the tenure of a Government of India scholarship and I am indebted to them for this help. Lastly, I acknowledge the help that Mr. Nath has rendered me by taking the photomicrographs.

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* Not seen by the author.

Explanation of Text-Figures

(All figures are from camera lucida sketches)

Text-Figs. 1–12. Fig. 1. Transverse section of a part of a rhizomorph showing the tissue of a young sporophyll and single large superficial ligule initial. Fig. 2. Same as above showing the ligule initial divided into a filament of three cells, the end cell of which is divided into two. Fig. 3. A later stage where the initial has divided into a mass of cells, the tissue of the ligule. Fig. 4. Longitudinal section of a young megasporophyll showing the ligule and below it a row of superficial sporangial initials. Fig. 5. Longitudinal section of a slightly older megasporophyll showing the sporangial initials divided into 2 rows of cells. The upper terminal cell forms the velum initial. Fig. 6. Longitudinal section of a still older megasporophyll showing velum initial divided into 3 cells. Fig. 7. An older megasporophyll longitudinally cut showing the earlier development of the velum and the sporangial initials still in dividing stage. Fig. 8. Longitudinal section of a young sterile leaf to show that only upper lobe of velum develops in the absence of sporangium. Fig. 9. Longitudinal section of a megasporophyll to show ligule and the velum with its tracheidal tissue. Fig. 10. Transverse section of rhizomorph cutting at the base of a sporophyll showing the sporangial mass of cells. Fig. 11. Longitudinal section of megasporophyll showing a megasporangium in which outermost layer has become differentiated from the sporangial tissue into an epidermis. Fig. 12. Longitudinal section of megasporophyll showing an older megasporangium with its epidermis and the sporangial tissue.

All sketches are magnified 240 times except Fig. 9 which is magnified 113 times.

Lettering.—ep., epidermis; *l.*, young leaf; *li.i.*, ligule initial; *lig.*, ligule; *rh.*, rhizomorph; *sp.i.*, sporangial initials; *spl.*, sporophyll; *sp.m.*, sporangial mass; *tr.t.*, tracheidal tissue; *vel.*, velum; *v.i.*, velum initial; *v.t.*, vascular tissue.

Text-Figs. 13–28. Figs. 13–16. Transverse section of megasporangium showing megaspore mother cells, isolated Fig. 13, in groups Fig. 14, in rows Fig. 15, and in both groups and rows Fig. 16. In Fig. 16 can be seen some megaspore mother cells dividing to produce sterile cells which eventually become trabeculæ or tapetum. Fig. 17. A group of megaspore mother cells with one central fully-developed megaspore mother cell, surrounding this are smaller megaspore mother cells; one of which is dividing to form sterile cells. Fig. 18. Transverse section of a megasporangium showing one megaspore mother cell dividing to form four sterile cells. Fig. 19. A megaspore mother cell divided into two; one of the daughter cells is dividing further and shows the metaphase stage with the chromosomes in the equatorial plane of the spindle. Cells surrounding these are sterile cells. Fig. 20. A megaspore mother cell showing nucleus situated slightly towards one side and a crescent-like body surrounding the nucleus and closely applied to it, *i.e.*, the polar body. Fig. 21. A megaspore mother cell showing nucleolar budding. Fig. 22. A megaspore mother cell showing the polar body constricted in the middle. Fig. 23. A megaspore mother cell, showing the two polar bodies which lie opposite to each other. Fig. 24. A megaspore mother cell, showing a second constriction of each of the polar bodies. Fig. 25. A megaspore mother cell in which the nucleus has become triangular having rod-like chromosomes. Polar bodies lie opposite each angle of the nucleus. The shrunken megaspore mother cell wall is also seen. Fig. 26. Transverse section of a megasporophyll showing the sterile tissue of the sporangium, breaking up to form the cavity of the sporangium. Figs. 27–31 are the megaspore mother cells showing different stages of division in the formation of spores. In all these stages megaspore mother cells lie freely in the megasporangial cavities. Fig. 27. Late prophase stage nuclear wall dissolves, chromosomes lie freely in the centre, polar bodies are seen at three corners. Fig. 28. Heterotypic metaphase, polar bodies arranged roughly in pairs. Chromosomes lying, in the equatorial plane, and spindle fibres, arising from in between the two polar bodies, are seen. Figs. 13–16 and 18, ×113; Figs. 17 and 19–25, ×583; Fig. 26, ×75; Figs. 27–28, ×583.

Lettering.—con., constriction; *chr.*, chromosomes; *ep.*, epidermis; *m.m.c.*, megaspore mother cell; *n.w.*, nuclear wall; *nuc.*, nucleus; *nucl.*, nucleolus; *p.b.*, polar body; *s. cav.*, sporangial cavity; *sp.*, sporangium; *st.t.*, sterile tissue; *v.t.*, vascular tissue; *w.*, megaspore mother cell wall.

TEXT-FIGS. 29–43. Fig. 29. Earlier anaphase stage. Chromosomes moving away from the equatorial plane to the poles. Fig. 30. Late anaphase stage. Chromosomes arranged at the poles in two groups and the spindle fibres stretched between the poles. Fig. 31. Cell plates between the daughter nuclei in the megaspore mother cell. Fig. 32. Transverse section of megasporophyll showing megasporangium with 3 spore tetrads lying free in the sporangial cavity into which trabeculæ are projecting. Dark shading shows the tapetum lining the sporangial cavity. Fig. 33. A portion of sporangial wall showing the disorganising tapetum. Fig. 34. Spores grouped in a tetrahedral form. Fig. 35. Spores grouped in a bilateral form. Fig. 36. A megaspore mother cell splitting all along the separating walls, but spores are still grouped inside the megaspore mother cell wall. Fig. 37. Two young spores attached to each other. Fig. 38. An older stage of the same kind of attachment. Fig. 39. Separated spores, showing sharp ridges formed along the triradiate mark and also the three-layered spore wall. Figs. 40 and 41. Rhizoid bearing gametophytic tissue coming out of the spores. Fig. 42. Young seedling with a single root and single leaf and also initiation of second leaf and second root. Vascular trace is a single strand of xylem slightly constricted at the junction of leaf and root. The gametophytic sheath is also seen. Fig. 43. Older seedling with second leaf and second root fully formed. Vascular supply to the second leaf and root comes from the constriction.

Figs. 29–31, ×583; Fig. 32, ×50; Fig. 33, ×240; Figs. 34–36 and 39, ×583; Figs. 37–38 and 40–43 ×50.

Lettering.—con., constriction; *cpl.*, cell plate; *ep.*, epidermis; *g.t.*, gametophytic tissue; *f.l.*, first leaf; *f.r.*, first root; *nuc.*, nucleus; *p.b.*, polar body; *s.cav.*, sporangial cavity; *s.l.*, second leaf; *s.r.*, second root; *s.h.*, gametophytic sheath; *spl.*, sporophyll; *spr.t.*, spore tetrads; *tap.*, tapetum; *trab.*, trabecula; *v.t.*, vascular tissue; *w.*, megaspore mother cell wall.

EXPLANATION OF PHOTOGRAPHS

(All photographs are from untouched negatives)

PLATE IV

Isætes sampathkumarani Rao, L. N.

PHOTO 1. Longitudinal section of a plant showing sporophylls cut in various planes. The young sporophyll on the left shows *lig.*, ligule; *sp.*, young sporangium; *vel.*, velum.

PHOTO 2. A megaspore mother cell (*m.m.c.*) surrounded by the tissue of the megasporangium; *nuc.*, nucleus; *nucl.*, nucleolus; *p.b.*, polar body.

PHOTO 3. A megaspore mother cell showing the heterotypic metaphase stage. *chr.*, chromosomes; *p.b.*, polar body.

PHOTO 4. A megaspore mother cell showing the cell plate (*c.pl.*) between four daughter nuclei along the equatorial plane; *p.b.*, polar body; *w.*, shrunken wall of megaspore mother cell.

PHOTO 5. Transverse section through megaspore showing *epi.*, an outer glassy colourless layer epispore; *Exo.*, middle exospore composed of *o.l.*, outer layer; *i.l.*, inner layer; and *end.*, innermost endospore; *r.*, a sharp ridge on one side.

PLATE V

PHOTO 6. Dimorphic megaspores from the same sporangium.

PHOTO 7. Both the above kinds of spores germinating and showing knob-like gametophytic tissues coming out of them.

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vel.
op.

epi
end
exo { il
ol
epi
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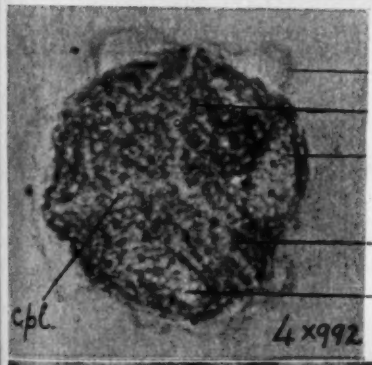
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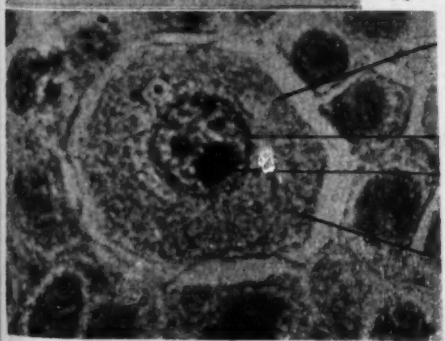
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chr.
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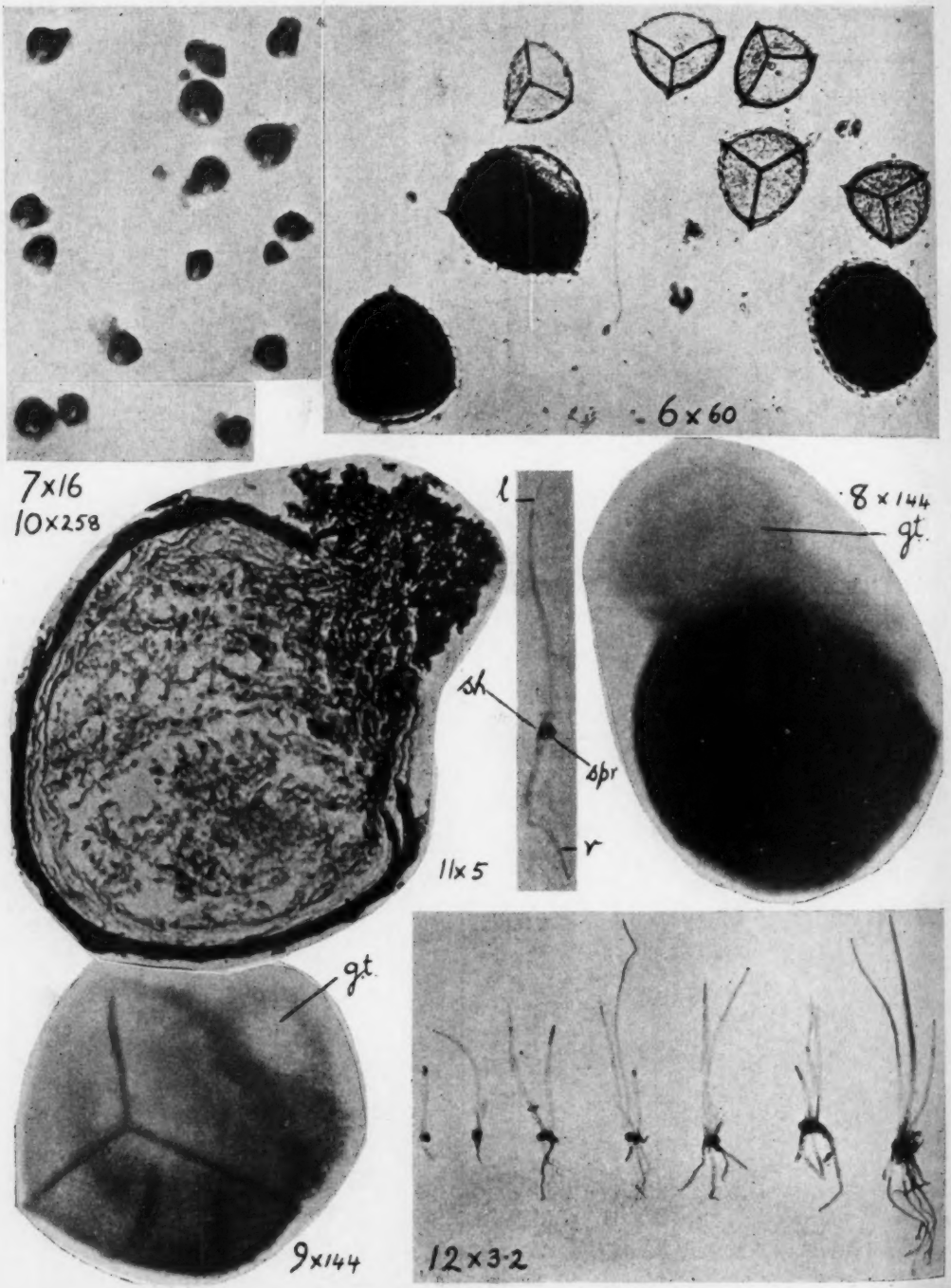
PHOTOS 1-5



PHOTOS 6–12

PHOTO 8. A ruptured megaspore showing the gametophytic tissue issuing out of a spore.

PHOTO 9. A megaspore showing the triradiate mark and the gametophytic tissue coming out of the spore from the other side, which is ruptured. *g.t.*, gametophytic tissue.

PHOTO 10. Longitudinal section of a megaspore showing gametophytic tissue arising from it, the thick wall of the megaspore and the loose and partly imperfectly organised tissue inside the spore.

PHOTO 11. Young seedling with just one root (*r*) and one leaf showing the gametophytic sheath (*sh.*) and the megaspore remains (*spr.*) still attached.

PHOTO 12. Seedlings of different stages of growth. The rhizomorph is found roughly at the 5th stage while the one on the extreme right shows the dichotomising roots.

DIETETIC HEPATIC LESIONS

Influence of Protein Level and Vitamin B_2 Complex Deficiency on the Liver of Rats

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Number of publications have appeared on the production of experimental hepatic lesions in various species of animals by dietary means (Rich and Hamilton, 1940; Lillie *et al.*, 1941, 1942; Blumberg and Grady, 1942; Webster, 1942; Gyorgy and Goldblatt, 1942; Himsworth and Glynn, 1944, 1944 *a* and *b*; Handler and Dubin, 1946; Jeff *et al.*, 1950). These lesions are preventable in many cases by an adequate addition of yeast, choline or other lipotropic agents to the basal diet, and in the case of hepatic necrosis by cystine, tocopherol (Gyorgy, 1949; Gyorgy and Goldblatt, 1949 and Himsworth and Lindon, 1949) and antibiotics (Gyorgy *et al.*, 1950). It has been stressed by many workers that the particular dietetic deficiency does not appear to affect the ultimate fibrosis and in fact most clinical cases of liver cirrhosis have a history not of a single isolated deficiency of a 'B' vitamin, or of protein or of a particular amino-acid but of longstanding and cumulative deficiencies of various dietary factors including vitamins of the 'B' complex and proteins.

The present investigations were designed to study the interrelationships between proteins and vitamins of the 'B' complex as they affect the liver.

Material and Methods

One hundred and sixty-eight healthy albino rats (Haffkine Inbred Strain) weighing between 35–48 gm. were distributed equally with reference to age, weight, sex and litter mates into eight groups. The composition of the diets is given in Table I.

In addition, all the animals of every group received daily 2 drops of cod-liver oil containing 1,000 I.U. of vitamin A and 100 I.U. of vitamin D per gm.

* An enquiry on the 'Role of Nutritional Factors in Hepatic Cirrhosis' under the auspices of the Indian Council of Medical Research, at the Haffkine Institute, Bombay, under Dr. M. V. Radhakrishna Rao.

TABLE I

Composition of the diets with supplements

Group No.	Casein %	Starch %	Sucorse %	Clarified butter %	Salt mixture* %	Supplement per rat per day
I	18	35	35	8	4	20 μg. of Thiamine
II	18	35	35	8	4	0·5 gm. of Brewer's yeast
III	10	39	39	8	4	20 μg. of Thiamine
IV	10	39	39	8	4	0·5 gm. of Brewer's yeast
V	8	40	40	8	4	20 μg. of Thiamine
VI	8	40	40	8	4	0·5 gm of Brewer's yeast
VII	5	41·5	41·5	8	4	20 μg. of Thiamine
VIII	5	41·5	41·5	8	4	0·5 gm. of Brewer's yeast

* Osborne and Mendell's Salt Mixture No. 185.

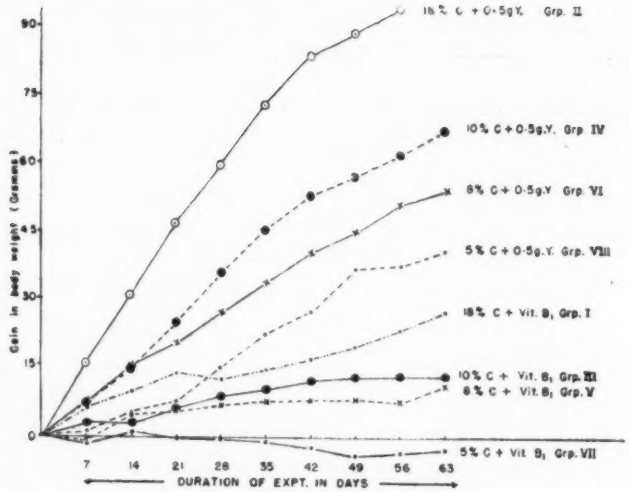
The food was supplied *ad libitum*, and the feeding of the diets was generally extended over many days, in certain cases up to 450 days. A number of animals from the deficient group however died early. In all cases, liver weight, its fat, protein and moisture contents were determined, following standard procedures.

Small portions of liver and kidney were taken for histological examination. All sections were cut to 6–7 μ thickness and stained by Ehrlich's acid hæmatoxylin and counterstained by 1 per cent. acloholic eosin. For collagen fibres Weigert's iron hæmatoxylin counterstained by Van Giesson's picric acid fuchsin was used. Reticulum fibres were studied by the silver impregnation method of Gomori.

RESULTS AND DISCUSSION

Graph I shows the growth curves of the animals.

Comparison of the growth curves (Graph I) of groups I, III, V and VII shows that animals of Group VII did not grow at all. They maintained their initial weight for some time and then gradually declined in weight. The



Graph I. Showing growth curves.

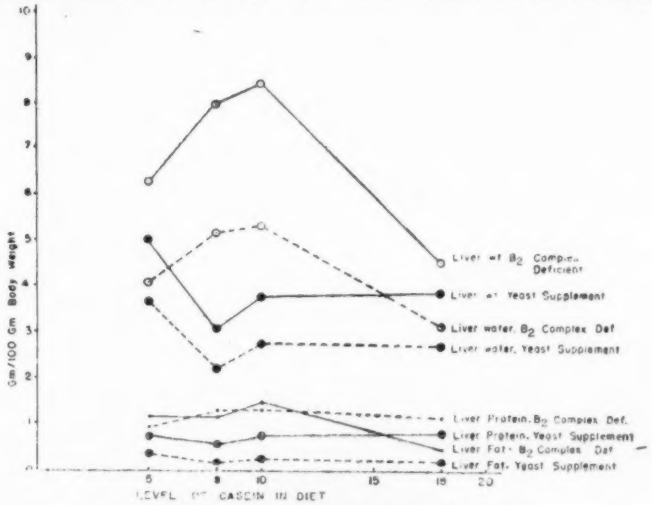
animals of the other three groups, *viz.*, V, III and I gained in weight and their growth was proportional to the level of casein in the diet. It is evident that at 5 per cent. casein (Group VII) level in the diet and in the absence of vitamin B_2 complex, the animals failed to grow. They also did not survive more than 100 days. Whereas, even when the diet is deficient in vitamin B_2 complex, increasing the level of protein in the diet brought about corresponding increase in the growth rate.

If Group VII and Group VIII are compared, it can be seen that though the animals of both the groups received protein at the same level (5 per cent. casein) in the diet, supplementation of the diet of Group VIII with vitamin B_2 complex brought about not only growth but also a remarkable increase in the growth rate. The animals of Group VIII survived as long as 400 days at the end of which period they were killed. Thus, it is observed that a very low protein diet when supplemented with vitamin B_2 complex promotes growth. Similar growth differences are also evident in other corresponding groups with and without yeast supplement.

Although the animals of Groups VIII, VI IV and II received the same quantum of vitamin B_2 complex, they showed a progressive increase in the growth rate as the level of protein (casein) in the diet was increased. This is so, for as the level of protein in the diet is increased greater quantum of protein is available from the diet for isogramme quantities of the different

diets for the growth of the animals. It has already been pointed out that for the same level of protein in the diet, the animals receiving vitamin B_2 complex showed marked increase in growth as compared to the animals deficient in vitamin B_2 complex. Hence it is clear that the rate of growth depends on the level of protein supplied and the adequacy of the vitamin B_2 complex in the diet.

From Graph II and Table II it can be seen that liver weight (g./100 g. body weight) of animals of Groups II, IV and VI, *i.e.*, those receiving dietary


Gm/100 Gm Body weight
Liver of B_2 Complex Deficient
Liver of Yeast Supplement
Liver water, B_2 Complex Def.
Liver water, Yeast Supplement
Liver Protein, B_2 Complex Def.
Liver Protein, Yeast Supplement
Liver Fat, B_2 Complex Def.
Liver Fat, Yeast Supplement
LEVEL OF CASEIN IN DIET

Graph II. Showing liver analysis values.

protein at 8 per cent. and higher levels with vitamin B_2 complex was within the normal range of 3 to 5 g./100 g. body weight. In Group VIII (casein 5 per cent. + B_2 complex), however, the liver weight was found to be slightly above the normal range. This could be attributed to the low protein level of the diet and consequent low protein intake resulting in higher moisture and lipid content.

In the vitamin B_2 complex deficient groups (Groups I, III, V and VII) the liver weight of the animals varied with the level of casein in the diet. It can be seen from Table II and Graph II that even though the animals of Group I were deficient in vitamin B_2 complex, yet as their intake of protein was at a higher level (18 per cent. in the diet), their liver weight was only 4·54 g./100 g. body weight, which is within the normal range. At 10 and 8 per cent. level of protein intake (Groups III and V respectively) the liver weight increased considerably. However when the protein intake was at

Liver weight, moisture, lipids and protein

TABLE II

Liver analysis for moisture, total lipids and protein
(Average values are expressed in g./100 g. body weight)

	Group No.	Liver			
		Weight	Moisture	Total lipids	Protein
Deficient in B_2 Complex ..	I	4·54	3·10	0·47	0·87
	III	8·45	5·32	1·45	1·30
	V	8·00	5·15	1·16	1·25
	VII	6·31	4·05	1·15	0·94
Receiving yeast supplement	II	3·85	2·75	0·17	0·79
	IV	3·82	2·81	0·19	0·73
	VI	3·10	2·22	0·14	0·61
	VIII	5·09	3·72	0·36	0·77

5 per cent. level (Group VII), the liver weight showed a decrease as compared to the 10 and 8 per cent. protein level groups. This may probably be due to inanition and the earlier death of the animals in this group. No significance can therefore be attached to the values obtained for this group for purposes of comparison. From the above findings it is observed that on a low protein intake, particularly in the absence of the members of the B_2 complex, there is an increase in the liver weight which is largely due to the summation effect of an increase in water and total lipid content of the liver.

Liver moisture and total lipids followed almost the same pattern as the liver weight described above for both the set of animals (*viz.*, Groups II, IV and VI; Groups I, III and V) except in the 5 per cent. casein-fed animals. The animals of Group VIII receiving vitamin B_2 complex showed slightly higher values for liver moisture and total lipid content whereas in animals deficient in vitamin B_2 complex (Group VII) the liver moisture and total lipid content had decreased. This was also corroborated by histological examination and may probably be due to the lowered protein intake in both the groups (Group VII and VIII) and inanition and shorter life-span of animals

particularly in Group VII. Liver protein values did not show much variation in animals of Groups II, IV, VI and VIII which received yeast supplement. Whereas in B_2 complex deficient animals 10 and 8 per cent. (Group III and V) casein-fed animals showed a higher liver protein value than the 18 and 5 per cent. (Groups I and VII) casein-fed groups. The lower liver protein content in 5 per cent. casein (Group VII) may be due to inanition.

A comparison of the liver total lipids of the groups of rats receiving casein at different levels in the diet in the presence and absence of the members of the B_2 complex may be interesting. Even while the dietary protein is as high as 18 per cent. casein, the absence of the members of the B_2 complex (Group I) increases the total lipids of the liver by nearly three times that of the corresponding 18 per cent. casein group receiving the vitamins of the B_2 complex (Group II). Under similar conditions, on a 10 and 8 per cent. casein diets, the difference in lipid deposition is nearly eight times. Addition of the members of the B_2 complex to a 5 per cent. casein diet (Group VIII) definitely decreases the total liver lipid content, but it is still higher than the normal values. These results of the effect of various levels of casein in a B_2 complex deficient diet are in general agreement with the previous reports (Gyorgy and Goldblatt, 1941; Webster, 1941; Handler and Dubin, 1946; Drill and Loomis, 1948) and show that both protein and the vitamins of the B_2 complex are important factors in the prevention of dietary fatty infiltration of the liver.

Histological Findings

The microscopic examination of liver sections showed that the liver parenchyma retained its normal histological structure in animals fed on 18 and 10 per cent. casein diets (Groups II and IV) with vitamins of the B_2 complex. Slight fatty infiltration of the parenchyma mostly round the central veins was seen in the livers of the animals which received 8 and 5 per cent. casein diets with vitamins of the B_2 complex (Groups VI and VIII). This fatty infiltration of the liver was more predominant in rats receiving the 5 per cent. casein diet (Group VIII). The liver sections of this group also showed a verified appearance of the cytoplasm, which was not seen in the other groups. Hepatic fibrosis was altogether absent in any of the groups of rats and its absence in the liver of the animals which received the low protein diets with the addition of yeast was particularly striking. It appears that yeast exerts an inhibitory effect on the development of hepatic fibrosis by virtue of its lipotropic activity. The observations of Himsworth and Glynn (1944 *a* and *b*) support these findings.

The microscopic examination of the liver sections of the rats from the four B_2 complex deficient groups (Groups I, III, V and VII) showed definite histological changes. Liver sections of rats receiving 18 per cent. casein diet (Group I) showed marked fatty infiltration of the parenchyma, particularly around the central veins. None of the animals of this group showed any signs of fibrosis. The 10 and 8 per cent. casein groups (Groups III and V) showed extensive fatty infiltration more marked around the central veins even as early as 70 days. Moderate replacement fibrosis, patchy in distribution around the central veins, was also common in these groups within 150 days. Necrosis of a few parenchymal cells around the central veins in other areas was seen along with dilated and congested sinusoids. The cells in these areas were more faintly stained than those around the portal veins. The areas showing marked fatty infiltration were represented at a later stage by dilated spaces in which a yellowish brown pigment-ceroid was seen.

Two animals from each of the 10 and 8 per cent. casein groups (Groups III and V) showed marked replacement fibrosis with a tendency for the formation of pseudolobules. Histological evidence favours the opinion that diets low in protein and deficient in the vitamins of the B_2 complex, as in these experiments, were more conducive for an earlier production of cirrhosis of the liver. On the 5 per cent. casein diet (Group VII), the liver sections showed varying degrees of fatty infiltration of the hepatic parenchyma and this was more marked around the central veins. Animals of this group did not show any marked degree of fibrosis. The animals of this group formed an exception as in their case the food intake was low and consequently the animals died at a very early period.

None of the animals in these experiments, which were on the low protein diets (Groups V and VII) however, showed acute massive hepatic necrosis, similar to that described by Himsworth and Glynn (1944 *a*, *b*).

The influence of yeast in the prevention of fatty livers may be due to the considerable amount of choline it contains (Himsworth *et al.*, 1944 *a*, *b*). Choline as well as casein possess lipotropic action, the effect of the latter being largely due to its methionine content (Tucker and Eckstein, 1937, 1938), which serves as a precursor of choline (du Vigneaud *et al.*, 1940, 1941). The lipotropic action of choline is particularly emphasised on a comparison of liver fat (*see* Table II) of groups of rats fed 5 per cent. casein with (Group VIII) and without (Group VII) yeast supplements and that of methionine on a comparison of liver fat of groups of rats receiving casein at levels of 18 per cent. (Group I) and 5 per cent. (Group VII) respectively in the absence of the vitamins of B_2 complex.

Summary

1. Attempts have been made to induce hepatic lesions in albino rats by varying the quantity of the protein (casein) and by withholding the supply of the vitamins of the B_2 complex.

2. On the vitamin B_2 complex deficient diets, the average life-span of the rats was largely influenced by the casein content of the diet, while those which received similar casein diets with yeast as supplement lived longer.

3. Fatty infiltration of the liver with progressive fibrosis (akin to human portal cirrhosis) can be more easily produced in rats by prolonged feeding of diets low in protein (casein) content and deficient in the vitamins of the B_2 complex.

4. Increasing the level of protein (casein) in the diet or supplementing low protein diets with yeast has a marked corrective effect on fatty infiltration of the liver and this is attributed to their respective methionine and choline content.

5. In general, the vitamins of the B_2 complex have a marked beneficial effect on the fatty livers when given early enough as a supplement.

Acknowledgement

Our thanks are due to the Director, Haffkine Institute, for his interest in this work, encouragement and facilities given; to Shri K. K. Govind, M.Sc., and Dr. S. M. Patel, Ph.D., for their suggestions.

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EXPLANATION OF PLATES

PLATE VI

Rat No. 11.—18 per cent. casein diet with vitamin B_1. Duration 84 days. Shows moderate fatty infiltration (Hæmatoxylin and Eosin, × 70).

Rat No. 163.—5 per cent. casein diet with vitamin B_1. Duration 63 days. Shows extensive fatty infiltration of the hepatic parenchyma (Hæmatoxylin and Eosin, × 70).

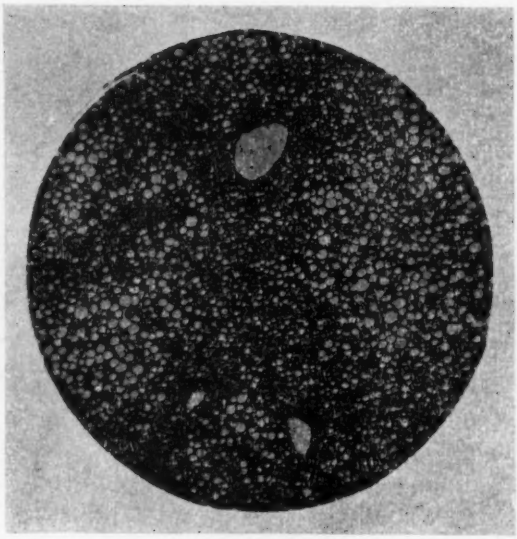
Rat No. 66.—10 per cent. casein diet with vitamin B_1. Duration 119 days. Shows fatty infiltration with marked ceroid pigmentation, (Hæmatoxylin and Eosin; × 70).

PLATE VII

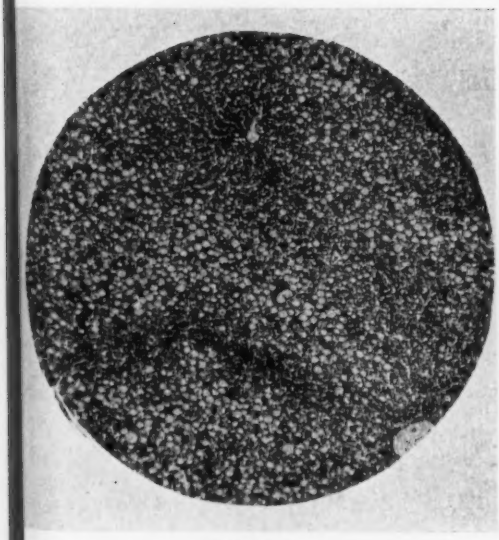
Rat No. 126.—10 per cent. casein diet with vitamin B_1. Duration 196 days. Shows fatty infiltration with well-marked connective tissue bands (Hæmatoxylin and Eosin, × 70).

Rat No. 126.—10 per cent. casein diet with vitamin B_1. Duration 196 days. Section stained with Gomori's silver impregnation method to delineate the reticulum fibres (Gomori's silver technique, × 140).

Rat No. 150.—8 per cent. casein diet with vitamin B_1. Duration 119 days. Shows fatty change and replacement fibrosis well distributed with a tendency for the formation of pseudolebules (Hæmatoxylin and Eosin, × 70).



RAT 11



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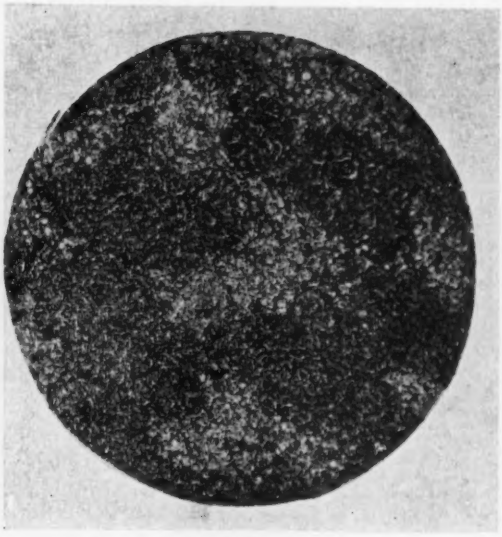

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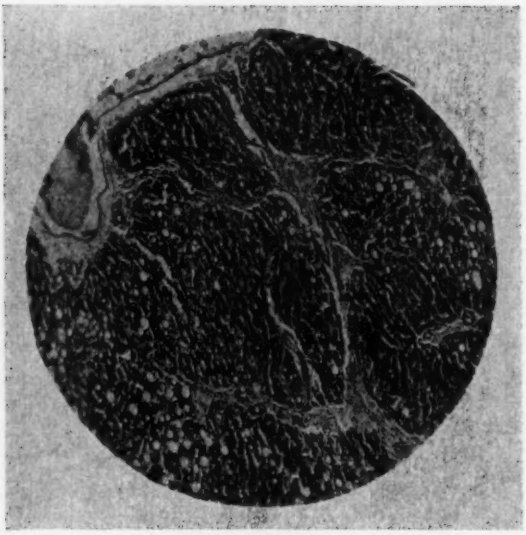

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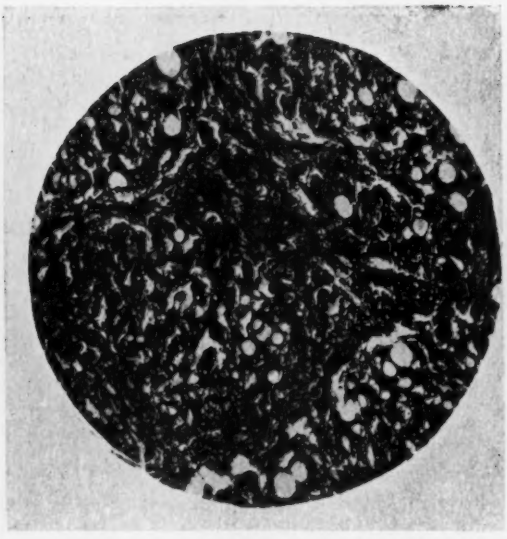

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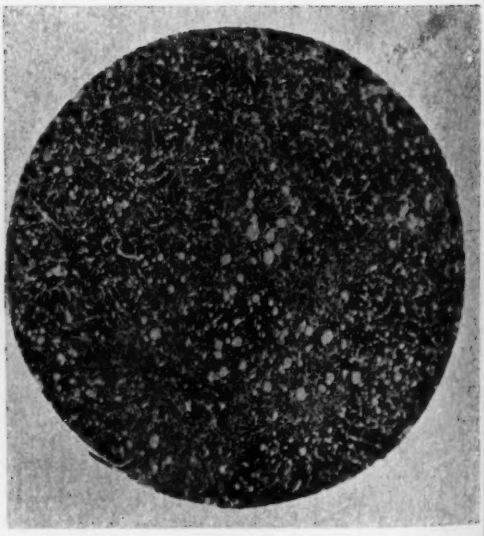

RAT 150

STUDIES ON THE MICROBIAL SPOILAGE OF CANNED FOOD

I. Isolation and Identification of Some Spoilage Bacteria

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Though canning industry in India is in its juvenile stage it is bound to grow up into an important one in coming years but as in other countries microbial contaminants would be a limitation to its development. Intense research work in the Western countries on the characters of the micro-organisms and on the methods of checking them has resulted in developing effective sterilization processes which are widely adopted by the industry. Because of the widely varying qualities of the microbial contaminants met with, it becomes imperative to adopt a method according to the contaminant. Also these methods vary according to the quality of the food product that is canned as it is the desire to preserve the maximum flavour and taste contained in the fresh food. The food material we deal with in India varies considerably from that in other countries. Also, because of the prevalent climatic conditions in this country the micro-organisms which contaminate the food products, either in the field or in the market place or in the industry are also likely to be different. It is therefore evident that we have to investigate into the details of the micro-organisms of canned food in India so as to evolve a method suitable under our conditions for effectively checking the damage.

Recently Subba Rao and Johar (1958) studies the micro-organisms contaminating Indian preserves or 'morabbas' and isolated 38 cultures of *Saccharomyces* belonging to *S. rouxii*, *S. nœllis* and *S. fermenti*. But no attempt seems to have been made so far to study the microbiology of canned food in India. Investigations were undertaken by the authors on examination of canned food for microbial population and on establishing their harmful effects and the results are reported here.

Material and Methods

Company-made cans of fruit and vegetable products showing apparent symptoms of spoilage were collected from groceries in Chidambaram,

Pondicherry and Madras during June-July, 1958. Also spoiled cans from one-year old stocks prepared at the Annamalai University Fruit Preservation Laboratory were selected for the studies (Fig. 1). The details for the samples finally selected for the studies are furnished in Table I.

TABLE I

Particulars for the spoiled cans used in these studies

Sl. No.	Name of product	Collected at	Age of the product	Size of can
			Years	
1	Sweet cherries	Pondichery	2	A 2½
2	Bartlett pears	Madras	1	A 2½
3	Canned potatoes	Annamalainagar	1	A 2
4	Tomato juice	Madras	2	A 2½
5	Fruit salad	Madras	2	A 2½
6	Mango sweet chutney ..	Annamalainagar	1	A 2
7	Pine apple	Chidambaram	2½	A 2½

The surface of the can was first washed thoroughly with water and dried. The lid was cleaned well with rectified spirit. Subsequently 5 ml. of spirit was sprinkled on the lid and ignited so as to sterilize the surface. Then the pointed end of the stem of the Pressure Gauge (Metal Box and Co., Ltd., India) and its rubber gasket were dipped in spirit and then pierced into the can through the sterilized surface, and the internal pressure noted. Then the hole was widened with a long pointed iron rod previously sterilized in flame. One ml. samples of the contents were drawn out from the can by inserting a sterile pipette in various directions. These samples were assayed for microbial population by the dilution plate method and the microbial colonies developing in the plates isolated and subcultured on agar slants.

The cans were also opened completely and a regular cut-out test was made as per the recommendations of the Central Food Technology Institute, Mysore (Personal communication).

For testing the harmful effects of bacteria on canned food, curried brinjal, sliced potato and mixed fruit with pear, pine apple, guava, banana, Manda-

rine and ber with cane sugar syrup added to give 50° Brix were prepared in the manner recommended by the Central Food Technology Institute, Mysore (Anonymous, 1956), and the bacterial cultures inoculated aseptically and the cans sealed in double seamer.

The bacterial isolates were studied for their morphological, cultural and physiological properties following the procedure described by Dowson (1957).

For studying the utilization of various carbon sources by the bacteria a basal medium containing ammonium hydrogen phosphate 1·0 gm., potassium chloride 0·2 gm., magnesium sulphate 0·2 gm., in a litre of distilled water (final pH 6·8) was prepared and divided into small quantities to which one per cent. W/V of various sources of carbon were added. The medium was then distributed in 10 ml. portions in test-tubes and autoclaved at 10 lb. pressure for 15 minutes. The tubes were inoculated with bacterial isolates grown in the above basal medium together with one per cent. dextrose and were incubated at room temperature.

To find out the capacity of the bacteria to utilize various nitrogen sources a basal medium containing dextrose 10·0 gm., potassium hydrogen phosphate 2·0 gm., magnesium sulphate 0·2 gm., and potassium chloride 0·2 gm. in a litre of distilled water (final pH 6·8) was prepared and the various nitrogen sources added at the rate of 0·5 per cent. W/V and the tubes prepared in manner described above. The bacterial cultures grown in the above basal medium together with 0·1 per cent. sodium nitrate were used as inocula.

Experimental Results

Isolation of micro-organism.—In all, seven fruit and vegetable cans were examined and the details of the cut-out tests on them are summarized in Table II. All the seven cans gave microbial colonies and only one type of colonies was observed in each of the cans. These organisms were brought into culture, purified by the single colony isolation method and used for further investigations (Fig. 2).

Test for spoilage.—The above mentioned isolates were tested for their harmful effects on the food products by inoculating freshly prepared and sterilized fruit and vegetable products. On inoculation the cans were sealed and incubated at room temperature for four months, on the expiry of which they were examined for microbial population, food quality, odour, etc., and the results are summarized in Table III.

All the seven isolates proved to be harmful, to a greater or lesser degree, o either the vegetable products or to both the vegetable and fruit products.

TABLE II

Some results of cut-out tests made on spoiled cans

Sl. No.	Name of product	External appearance	Internal appearance	Internal pressure (lb.)	Taste and smell	% Total soluble solids	Bacterial population
1	Sweet cherries	Swell	Bright	11·0	Normal	22	Bacteria present but population not estimated
2	Bartlett pears	Springer	Corrosive	4·5	Normal	17	do.
3	Canned potatoes	Swell	Bright	13·0	Disagreeable	6	do.
4	Tomato juice	Springer	Blackening	4·5	Ferric smell	11	1·48 million/ml.
5	Fruit salad	Swell	Corrosive	19·5	Disagreeable	24	5·65 million/ml.
6	Mango sweet chutney	Swell	Corrosive	16·5	Acidic smell	19	Bacteria present, but population not estimated
7	Pine apple	Swell	Corrosive	19·0	Ferric smell	18	0·985 million/ml.

The damage was more in the curried vegetable and potato than in the mixed fruit.

Morphological and cultural characters.—The details of the morphological and cultural characters of the 7 isolates are summarized below:—

Isolate 1.—Long rod, single or in pairs, 0·9–1·2×2·3–6·8 μ, Gram-positive; flagellate with peritrichous flagella, capsulate, sporulate with 0·4–0·8×1·65–1·9 μ ellipsoidal central spores; growth on nutrient agar translucent with spreading colonies, in nutrient broth turbid with sedimentation, on potato plug warty, slimy and yellowish-brown; infundibuliform liquefaction of gelatin, coagulation and acid formation in litmus milk; hydrolysis of starch and reduction of nitrate; positive M.R. test and negative indole, NH_3, H_2S and V.P. tests.

Isolate 2.—Long rods, single or in pairs, 1·0–1·2×1·6–6·2 μ; Gram-variable; flagellate with peritrichous flagella, capsulated, sporulate with

TABLE III

The changes caused by bacterial isolates on freshly canned sterile food products: cut-out four months after inoculation

Isolate No.	Food product tested and the damage caused		
	Canned vegetable	Canned potato	Canned mixed fruits
1	Swell; putrefaction; internal pressure 11 lb.; bacterial population 13·5 mil./ml.	Swell; contents dull yellow and cloudy; internal pressure 16 lb.; bacterial population 7·1 mil./ml.	No swell but the contents putrefied and dark-brown; not much of gas formation.
2	No swell but putrefaction; internal pressure 3·5 lb.; bacterial population 9·4 mil./ml.	No swell; contents yellow; not much of gas; bacterial population 1·2 mil./ml.	No swell; contents soft and brown; internal pressure 3·5 lb.
3	Swell; contents pasty; internal pressure 9·5 lb.; bacterial population 76·5 mil./ml.	Swell; contents disintegrated and straw coloured; internal pressure 8·5 lb; bacterial population 2·47 mil./ml.	No swell; contents dark-brown and putrefied; internal pressure 3 lb.; bacterial population 6·7 mil./ml.
4	Swell; putrefaction; internal pressure 14 lb.; bacterial population 17·5 mil./ml.	Flipper; contents disintegrated and brown; internal pressure 5 lb.; bacterial population 6·1 mil./ml.	Springer; contents frothing and dark-brown; internal pressure 6·5 lb.; bacterial population 3·6 mil./ml.
5	Swell; contents pasty; internal pressure 7·5 lb.; bacterial population 137 mil./ml.	Swell; contents disintegrated and straw coloured; internal pressure 9 lb.; bacterial population 15·2 mil./ml.	No swell but contents frothing and dark-brown; internal pressure 2 lb.
6	Swell; putrefaction; internal pressure 13·5 lb.; bacterial population 4·1 mil./ml.	Swell; contents disintegrated and dull yellow; internal pressure 11 lb.; bacterial population 2·7 mil./ml.	Swell; putrefaction; contents dark-brown; internal pressure 11·5 lb.; bacterial population 1·4 mil./ml.
7	Swell; contents pasty; internal pressure 22·5 lb.; bacterial population 57 mil./ml.	Springer; contents disintegrated and dull yellow; internal pressure 7·5 lb.; bacterial population 26·3 mil./ml.	No swell but contents frothing and dark-brown; internal pressure 2·5 lb.

ellipsoidal to sub-terminal 0·6–0·8×1·5–1·7 μ spores; growth on nutrient agar translucent with undulate spreading colonies, in nutrient broth turbid with sedimentation, on potato plug spreading and creamy; crateriform liquefaction of gelatin; slight digestion and acid formation of litmus milk; starch not hydrolysed but nitrate reduced; positive M.R. test and negative indole, NH_3, H_2S and V.P. tests.

Isolate 3.—Short rods, single or in pairs, 0·7–0·9×1·8–4·0 μ; Gram-positive; flagellate with peritrichous flagella; capsulated, sporulate with ellipsoidal to cylindrical 0·6–0·8×1·1–1·5 μ central spores; growth on nutrient agar matty, lacerate with spreading colonies, in nutrient broth turbid with pellicle formation, on potato plug brown and spreading with wrinkles; crateriform liquefaction of gelatin; peptonization of litmus milk but no acid formation; starch hydrolysed and nitrate reduced; positive M.R. and V.P. tests and negative indole, NH_3 and H_2S tests.

Isolate 4.—Short rods, single or in pairs, 0·4–0·6×3·0–4·0 μ; Gram-positive; non-flagellate, non-capsulate and no spore formation; growth on nutrient agar translucent, circular and non-spreading, in nutrient broth turbid, on potato plug light brown and spreading; slight liquefaction of gelatin; acid formation and reduction of litmus in litmus milk; no hydrolysis of starch and nitrate not reduced; positive indole and H_2S tests and negative NH_3, M.R. and V.P. tests.

Isolate 5.—Long rods, single or in pairs, 1·0–1·2×3·0–7·2 μ; Gram-positive; flagellate with peritrichous flagella, capsulate, sporulate with 0·4–0·6×1·9–2·1 μ sub-terminal spores; growth on nutrient agar tiny with discrete colonies, in nutrient broth turbid with sedimentation, on potato plug matty and dull grey; rapid saccate liquefaction of gelatin, slight digestion without acid formation of litmus milk; starch hydrolysed but nitrate not reduced; positive M.R. test and negative indole, NH_3, H_2S and V.P. tests.

Isolate 6.—Short rods, single or in pairs, 0·5–0·9×2·0–5·2 μ; Gram-positive; flagellate with peritrichous flagella, capsulate, sporulate with 0·4–0·6×1·2–1·4 μ sub-terminal spores; growth on nutrient agar opaque undulate and spreading, in nutrient broth turbid with pellicle formation, on potato plug matty and dull grey; napiform liquefaction of gelatin; slight digestion, with acid formation of litmus milk; starch hydrolysed but nitrate not reduced; positive M.R. and V.P. tests and negative indole, NH_3 and H_2S tests.

Isolate 7.—Short rods, single or in pairs, 0·6–0·8×1·5–4·8 μ; Gram-positive; flagellate with peritrichous flagella, non-capsulate, sporulate with

0·4–0·6 × 1·0–1·4 μ ellipsoidal to cylindrical central spores; growth on nutrient agar matty, rhizoidal and quick spreading, in nutrient broth turbid with pellicle formation, on potato plug reddish, heavy spreading; stratiform liquefaction of gelatin; hydrolysis of casein with acid formation in litmus milk; starch hydrolysed and nitrate reduced; positive M.R. and V.P. tests and negative indole, NH_3 and H_2S tests.

Utilization of carbon sources.—The bacterial isolates were then tested for their capacity to utilize various carbon sources for their growth. The experiment was conducted in triplicates and the results are summarized in Table IV.

Out of the thirteen carbon sources tested isolates 1 and 2 utilized all of them, isolate 3 utilized 11 sources, isolate 4 eight, isolate 5 seven, isolate 6 nine and isolate 7 ten carbon sources, respectively.

Utilization of nitrogen sources.—The bacterial isolates were examined for their capacity to utilize two organic and two inorganic nitrogen sources, following the method described under Material and Methods. The results are summarized in Table V.

All the four nitrogen sources were utilized by the isolates 2, 3, 4, 5 and 6, while no growth was recorded in ammonium chloride by isolate 1 and in ammonium chloride and leucine by isolate 7.

Identity of the isolates.—The isolates were classified on the basis of their morphological, cultural and physiological properties following the system given in the Seventh Edition of Bergey's *Manual of Determinative Bacteriology* (Breed *et al.*, 1957). Six of the isolates, Nos. 1, 2, 3, 5, 6 and 7, formed endospores which were ellipsoidal to cylindrical; the sporangia were not different from the vegetative cells except when bulged by the spores and so they are classified under the family Bacillaceæ Fischer. One of the six isolates, No. 5, was found to be anaerobic, aerotolerant and proteolytic and so is classified under the genus *Clostridium* Prazmowski and because of its flagellate cells, distinctly swelled sub-terminal endospores, capacity to liquify gelatin very quickly and not fermenting carbohydrates, the isolate is further classified as *Clostridium histolyticum* (Weinberg and Senguin) Bergey *et al.*

The other five spore formers are all aerobic with ellipsoidal to cylindrical spores and so are classified under the genus *Bacillus* Cohn. Of these, isolate 1 is identified as *B. circulans* Jordan because of its thick-walled spores, capacity to hydrolyse starch and produce acid from arabinose. Isolate 2 is identified as *B. brevis* Migula, since it was distinct in not hydrolysing starch and not producing acid from arabinose and lactose but giving a cream-coloured growth on potato plug. Isolate 3 produced acid from arabinose, glucose,

TABLE IV

Utilization of carbon sources by the bacterial isolates from spoiled fruit and vegetable cans

C-source	Isolate No.						
	1	2	3	4	5	6	7
Arabinose ..	+++ A	+ NA	+++ A	0	+++ SA	+ NA	+++ SA
Glucose ..	+++ A	+++ A	+++ A	+++ A	++ SA	++ SA	+++ SA
Galactose ..	+ NA	++ NA	+ NA	+++ SA	0	+ NA	+ NA
Mannose ..	+ NA	++ NA	+ NA	++ SA	+++ NA	+ NA	+ NA
Lactose ..	+ NA	+ SA	+ NA	+++ SA	+ NA	+++ SA	+ SA
Sucrose ..	+++ A	+++ A	+++ SA	+++ A	+++ SA	+++ A	++ SA
Maltose ..	+++ SA	+ NA	++ SA	++ SA	0	++ NA	+ NA
Raffinose ..	+ NA	++ SA	+ NA	+ SA	0	0	0
Starch ..	+++ A	++ NA	+++ A	+ NA	+++ SA	+++ A	++ A
Glycerol ..	+ NA	+ NA	0	0	0	+ SA	0
Mannitol ..	+ NA	+++ SA	+++ A	0	+ NA	0	++ SA
Sorbitol ..	++ NA	+ NA	+ NA	0	0	0	0
Salicin ..	+ NA	+ NA	0	0	0	0	+ NA

0 to + + + = increasing amount of growth; A = acid production; SA = slight acid production; NA = no acid production.

TABLE V

Utilization of some nitrogen sources by the bacterial isolates

Nitrogen sources	Isolate No.						
	1	2	3	4	5	6	7
Sodium nitrate	+++ SA	+ NA	+ NA	+ NA	++ SA	++ NA	+ SA
Ammonium chloride ..	0	+ NA	++ A	+ NA	++ SA	+ NA	0
L-asparagin	++ SA	++ A	++ A	++ SA	++ SA	++ SA	++ NA
L-leucine	++ NA	++ SA	++ SA	++ SA	+++ SA	++ SA	0

0 to + + + = increasing amount of growth; A = acid production; SA = slight acid production; NA = no acid production.

sucrose and mannitol and peptonized milk and so is identified as *B. subtilis* Cohn. Isolate 6 is identified as *B. coagulans* Hammer because of its highly thermophilic nature, small-sized cells and capacity to produce acid from arabinose, lactose, sucrose and mannitol but not to reduce nitrate. Isolate 7 was highly saccharolytic, hydrolysed casein and produced acid from glucose, lactose and mannitol and so is identified as *B. licheniformis* (Weighmann) Chester.

Isolate 4 was distinct from the rest in possessing long non-sporing rods, without flagella. It produced droplet-like colonies on agar media and utilized mono and disaccharides, except arabinose and did not reduce nitrate. Hence it is identified as *Lactobacillus fermenti* Beijerinck.

DISCUSSION

In the present studies both spore formers and non-spore formers were found to cause damage to the food products. The inoculation studies on freshly prepared sterile food products have shown that all the seven organisms under investigation were harmful to a lesser or greater extent. All of them, except isolate 2, caused swelling of the cans, but isolates 5 and 7 seem to be more destructive in this respect.

The organisms isolated seem to be somewhat different from the usual types reported in other countries. *Bacillus subtilis*, *B. coagulans* and *B. circulans* have been isolated from spoiled canned food in other countries (Tanner, 1944; Jones, 1949; Gordon and Smith, 1949; Allen, 1953), but *B. licheniformis*, *B. brevis* and *Cl. histolyticum* have not been found associated with the spoilage in other countries. Probably they are widely prevalent under Indian conditions, but a thorough survey is necessary.

Judging from the properties of the contaminants it could be said that they had entered the cans either due to defective processing or they had survived the process because of their capacity to withstand the treatments. The non-sporing rod, *L. fermenti*, is thermolabile and so is easily destroyable by the usual canning process. It is therefore probable that it had gained entrance into the can because of the under processing. Similarly, *Cl. histolyticum* is relatively less resistant to heat and so might have survived in the cans due to defective processing. The spore forming bacteria, on the other hand, are more difficult to check because of their resistance to heat treatment. Detailed investigations on their heat resistance qualities and susceptibility to chemical preservatives would help in combating them.

The capacity of the six of the isolates to utilize a wide range of sugars seems to indicate their dangerous effect on the food material. In this respect isolate 2 was found to be less harmful as it was more exacting in its nutritional requirements.

SUMMARY

In an attempt to study the microbial spoilage of canned food seven bacteria were isolated from seven different spoiled canned foods collected from various sources. When the pure cultures of the bacterial isolates were inoculated into freshly prepared and sterilized cans of curried vegetable, sliced potato and mixed fruit, all of them proved to cause harmful effects to a lesser or greater extent.

The bacteria were studied in detail for their morphological, cultural and physiological properties including their capacity to utilize certain carbon and nitrogen sources and on the basis of the results obtained they are identified as *Bacillus circulans* Jordan, *B. brevis* Migula, *B. subtilis* Cohn, *B. coagulans* Hammer, *B. licheniformis* (Weighmann) Chester, *Lactobacillus fermenti* Beijerinck and *Clostridium histolyticum* (Weinberg and Seguin) Bergey *et al*.

FIG. 1. Three types of samples of spoiled food cans collected from the groceries as compared to a sound one.

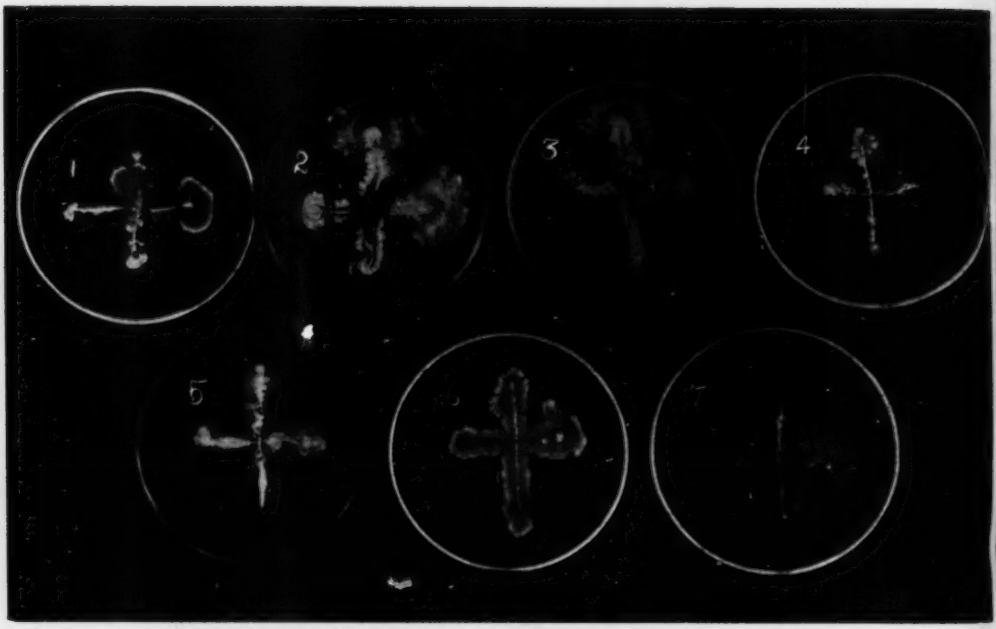

FIG. 2. The seven bacterial isolates from spoiled canned food, grown on nutrient agar.

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